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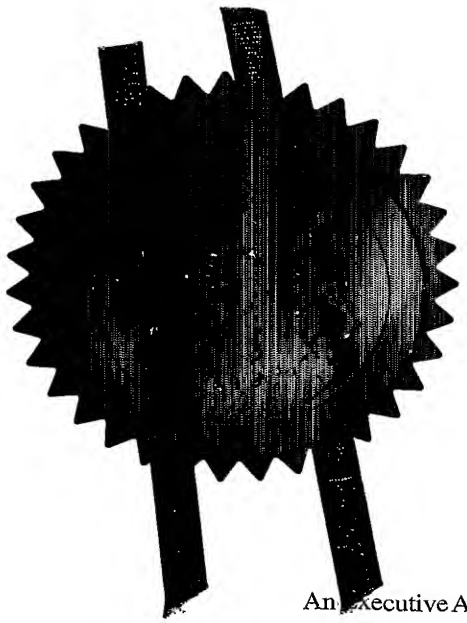

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Dated 14 March 2005

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Patents ADP number *(if you know it)*	07358700 3	
If the applicant is a corporate body, give the country/state of its corporation.	United Kingdom	
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Immunoglobulins

Field of the invention

The present invention relates to immunoglobulins that specifically bind Oncostatin M (OSM) and in particular human OSM (hOSM). More particularly, the present invention relates to antibodies that specifically bind hOSM. The present invention also concerns methods of treating diseases or disorders with said immunoglobulins, pharmaceutical compositions comprising said immunoglobulins and methods of manufacture. Other aspects of the present invention will be apparent from the description below.

Background of the invention

Oncostatin M is a 28 KDa glycoprotein that belongs to the interleukin 6 (IL-6) family of cytokines which includes IL-6, Leukaemia Inhibitory Factor (LIF), ciliary neurotrophic factor (CNTF), cardiotropin-1 (CT-1) and cardiotrophin-1 like cytokine. Of these, LIF is the family member most closely related to OSM at the genetic, structural and functional levels. OSM was originally discovered by its ability to inhibit the growth of the melanoma cell line A375. Subsequently, more effects were discovered and it was found to be a multifunctional mediator like other members of the IL-6 family. OSM is produced in a variety of cell types including macrophages, activated T cells, polymorphonuclear neutrophils, eosinophils, dendritic cells, some epithelial cells and bone marrow stromal cells. In most cases, OSM is an induced rather than constitutively expressed cytokine, the main exception being in bone marrow. Its principle biological effects include activation of endothelium, activation of

the acute phase response, induction of cellular proliferation or differentiation, modulation of inflammatory mediator release and haematopoesis, re-modelling of bone and promotion of wound healing.

Receptors for OSM are expressed on a wide range of cells including epithelial cells, chondrocytes, fibroblasts, neuronal smooth muscle and endothelial cells. Several lines of evidence suggest that endothelial cells are a primary target for OSM. These cells express 10 to 20 fold higher numbers of both high and low affinity receptors and exhibit profound and prolonged alterations in phenotype following stimulation with OSM. In addition, OSM is a major autocrine growth factor for Kaposi's sarcoma cells, which are thought to be of endothelial origin.

In common with other IL-6 family cytokines, OSM binds to the transmembrane signal transducing glycoprotein gp130. A key feature of the gp130 cytokines is the formation of oligomeric receptor complexes that comprise gp130 and one or more co-receptors depending on the ligand. As a result, these cytokines can mediate both the shared and unique biological activities *in vitro* and *in vivo* depending on the composition of the receptor complex formed. Human OSM (hOSM) differs from the other IL-6 cytokines in that it can form complexes with gp130 andeither one of the two co-receptors, LIFR or the oncostatin receptor (OSMR). Figure 1 illustrates the interaction between hOSM and gp130, LIFR and OSMR. The amino acid sequence for hOSM is set forth as SEQ.I.D.NO: 13

MGVLLTQRTLLSLVLALLFPSMASMAAIGSCSKEYRVLLGQLQKQTDLMQD
TSRLLDPYIRIQGLDVPKLREHCRERPGAFPSEETLRGLGRRGFLQTLNAT
LGCVLHRLADLEQRLPKAQDLERSGLNIEDLEKLQMARPNILGLRNNIYCM
AQLLDNSDTAEPTKAGRGASQPPTPTPASDAFQRKLEGCRFLHGYHRFMHS
VGRVFSKWGESPNRSRRHSPHQALRKGVRRTRPSRKGKRLMTRGQLP
R. (SEQ.I.D.NO: 13).

Site II residues of particular note are highlighted in bold and underlined

A cDNA encoding hOSM is set forth in SEQ.I.D.NO:14.

ATGGGGGTACTGCTCACACAGAGGACGCTGCTCAGTCTGGTCCTTGCACTC
CTGTTTCCAAGCATGGCGAGCATGGCGGCTATAGGCAGCTGCTCGAAAGAG
TACCGCGTGCTCCTTGGCCAGCTCCAGAAGCAGACAGATCTCATGCAGGAC
ACCAGCAGACTCCTGGACCCCTATATACGTATCCAAGGCCTGGATGTTCCT
AAACTGAGAGAGCACTGCAGGGAGCGCCCCGGGGCCTTCCCCAGTGAGGAG
ACCCTGAGGGGGCTGGGCAGGCGGGGCTTCCTGCAGACCCTCAATGCCACA
CTGGGCTGCGTCCTGCACAGACTGGCCGACTTAGAGCAGCGCCTCCCCAAG
GCCCAGGATTTGGAGAGGTCTGGGCTGAACATCGAGGACTTGGAGAAGCTG
CAGATGGCGAGGCCGAACATCCTCGGGCTCAGGAACAACATCTACTGCATG
GCCCAGCTGCTGGACAACTCAGACACGGCTGAGCCCACGAAGGCTGGCCGG
GGGGCCTCTCAGCCGCCCACCCCCACCCCTGCCTCGGATGCTTTTCAGCGC
AAGCTGGAGGGCTGCAGGTTCCTGCATGGCTACCATCGCTTCATGCACTCA
GTGGGGCGGGTCTTCAGCAAGTGGGGGGAGAGCCCGAACCGGAGCCGGAGA
CACAGCCCCCACCAGGCCCTGAGGAAGGGGGTGCGCAGGACCAGACCCTCC
AGGAAAGGCAAGAGACTCATGACCAGGGGACAGCTGCCCCGGTAG
(SEQ.I.D.NO:14)

The crystal structure of hOSM has been solved and shown to comprise a four α helical bundle with two potential glycosylation sites. Two separate ligand binding sites have been identified by site-directed mutagenesis on the hOSM molecule. The first, called Site II interacts with gp130 and the second site, called Site III, at the opposite end of the moleculeinteracts with either LIFR or OSMR. Mutagenesis experiments have shown that the binding sites for LIFR and OSMR are almost identical but that a single amino acid mutation can discriminate between the two. Based on this model the present inventors postulate that modulating (in particular blocking) the interaction of Site II and gp130 with an antibody against Site

II will modulate signalling by all of the potential OSM receptor complexes, effectively neutralising the biological activity of the cytokine when the OSM-gp130 interaction is blocked.

OSM is synthesised as a proprotein containing a hydrophobic 25 amino acid (AA) N termimal signal sequence and a C-terminal propeptide of 33 AA, both of which are cleaved to generate mature OSM. The OSM proprotein does have biological activity but this is significantly increased by cleavage of the C terminal propeptide (see Bruce A.G. *et al* (1992) Prog.Growth Factor Res. 4: 157-170, Malik N *et al* (1989) Mol.Cell Biol. 9: 2847-2853). OSM has been described as a "compact, barrel-shaped molecule" with dimensions of approximately 20Å x 27Å x 56Å. There are four alpha helical regions (helix A 10-37AA, helix B 67-90AA, helix C 105-131AA and helix D 159-185AA, numbering of AA starts after removal of the signal sequence). Helices A and C contain "kinks". The helices are joined by two overhand loops (AB loop 38-66AA, CD loop 130-158 AA) and are arranged as two anti-parallel pairs (A-D and B-C). (See Deller M.C *et al* (2000) Structure 8; 863-874).

It appears that OSM binding via Site II to gp130 and allows binding of another OSM molecule to gp130 by a Site III interaction. OSM will also bind to either LIFR or OSMR via Site III. Thus OSM forms a complex with its receptor consisting of; one gp130, one LIFR or OSMR, and two OSM molecules. (See Sporeno E (1994) J.Biol.Chem.269: 10991-10995, Staunton D *et al* (1998) Prot.Engineer 11:1093-1102 and Gearing D.P (1992) Science 225:306-312).

Using mutagenesis, the important residues for Site II OSM-gp130 binding are Gln20, Gly120, Gln16 and Asn124. For Site III OSM-OSMR binding, the important residues are Phe160 and Lys163. The OSM Site II

interaction is therefore dependent on Gln20, Gly120, Asn124 and to a lesser extent Gln16 on hOSM. Three complementary residues in gp130 (Phe168, Tyr196 and Glu282) have been identified as of particular note in the interaction between OSM and gp130. (See Aasland D *et al* (2002) J.Mol.Biol.315: 637-646, Timmermann A *et al* (2000) FEBS Lett.468: 120-124).

Rheumatoid arthritis (RA) comprises a syndrome of distinct but inter-connected pathogenic processes. These are: local and systemic inflammation, proliferation of synovial cells, angiogenesis and matrix deposition leading to formation of pannus tissue which invades and destroys cartilage and bone, resulting in deformity and disability. Underpinning this pathology is the chronic release of cytokines and inflammatory mediators from cells that enter and take up residence in the inflamed joint and from endogenous joint tissue cells. The initiating events in RA are unknown but a wealth of evidence suggests that they involve activation of T lymphocytes by either a foreign or autologous "self" antigen. The extent to which T cells are required to maintain the ongoing disease processes once they have been initiated is also uncertain although therapeutic agents such as CTLA4Ig, which specifically target T cells can be effective in advanced disease.

The earliest events in the development of rheumatoid synovitis involve recruitment of mononuclear and polymorphonuclear cells to cross the endothelium in capillaries in the synovial-lining layer. While the polymorphs migrate into synovial fluid (SF) the lymphocytes remain close to the capillaries and may subsequently become organised into ectopic lymphoid follicles. This influx of immune cells is followed by proliferation of fibroblast-like synoviocytes (FLS). Unlike their normal counterparts, RA FLS appear to have escaped from the regulatory processes that result in

arrest of proliferation and apoptosis leading to their continuing accumulation. Furthermore, the emerging pannus tissue now develops new blood vessels supported by extracellular matrix to allow further expansion. This process involving fibroblast proliferation, matrix – remodelling and angiogenesis closely resembles an uncontrolled wound-healing event. Monocytes migrate into the developing pannus tissue and undergo differentation into macrophages with a chronically activated phenotype. Similarly B cells undergo terminal differentiation to form long-lived plasma cells which secrete antibodies including rheumatoid factors. The ability of the inflamed synovium to sustain local differentation of myeloid and lymphoid cells is based, in part, on local production of growth factors such as GMCSF and IL-6. Both the FLS and resident mononuclear leukocytes release soluble factors that stimulate further recruitment of inflammatory cells from the blood and, critically, drive the next step in the disease process – the destruction of articular cartilage and re-modelling of bone. Pannus tissue is invasive. Its leading edge secretes destructive enzymes such as MMPs and cytokines that alter the phenotype of cells which maintain the structural integrity of cartilage and bone. As a result, proteoglycans are lost and type II collagen is irreversibly cleaved leading to weakening and loss of cartilage. Bone also undergoes a number of profound changes, which include focal erosions, sub-chondral osteoporosis and abnormal bone growth leading to osteophyte formation. Ultimately these changes result in the characteristic deformity and subluxation of the joints seen in advanced RA.

RA is a systemic disease probably as a result of the passage of inflammatory mediators from the joint into the blood. This affects many organ systems in the body including skin, eyes, liver, kidneys, brain and the vascular lining, leading to increased morbidity and mortality. Much of the excess mortality is due to cardiovascular disease caused by

atherosclerosis since many of the pathogenic processes involved in the development of rheumatoid synovitis are common to the formation of atherosclerotic plaques.

Treatments for RA aim to control pain, reduce inflammation and arrest the processes that result in tissue destruction. Traditionally RA has been treated with non-steroidal anti-inflammatory drugs (NSAIDS), low doses of steroids and so-called disease modifying anti-rheumatic drugs (DMARDS). Low levels of efficacy, slow onset, toxicity, poor tolerability and increasing resistance over time plague the use of these treatments which include methotrexate (MTX), sulphasalazine, gold and Leflunomide. More recently, the introduction of biologic drugs such as Enbrel™, Remicide™ and Humira™, which inhibit the cytokine Tumour Necrosis Factor (TNF), have been a significant advance.

It is therefore an object of the present invention to provide a therapeutic approach to the treatment of RA and other diseases and disorders, particularly chronic inflammatory diseases and disorders such as osteoarthritis and psoriasis. In particular it is an object of the present invention to provide immunoglobulins, especially antibodies that specifically bind OSM (e.g. hOSM, particularly Site II thereof) and modulate (e.g. inhibit or block) the interaction between OSM and gp130 in the treatment of diseases responsive to modulation of that interaction.

There is increasing evidence to support the hypothesis that modulating OSM-gp130 interaction maybe of benefit in the treatment of such diseases and disorders.

Clinical Evidence

OSM is found in the SF and at lower levels in the plasma of human RA patients. These levels correlate with; the number of neutrophils in SF, levels of TNF in SF, severity of synovitis and markers of cartilage destruction. Furthermore, the synovial tissue from RA patients secretes OSM spontaneously *ex vivo*. It has also been demonstrated that OSM is present in synovial macrophages and as discussed earlier, OSM receptors and gp130 are expressed on endothelial cells, synovial fibroblasts, chonodrocytes and osteoblasts. Furthermore, cells infiltrating atherosclerotic plaques and aortic aneurysms express OSM suggesting an association of this cytokine with chronic inflammation.

In Vitro evidence

Endothelial cells express ten to twenty times the number of OSM receptors than other cell types. OSM alone, or synergistically in combination with other cytokines, activates endothelium to release cytokines and chemokines and bind neutrophils, monocytes and lymphocytes mediating their extravasation into synovial tissue. OSM has also been demonstrated to be a potent stimulator of angiogenesis, FLS cells (thus facilitating the formation of pannus tissue, the release of IL-6, MMPs) and acts synergistically with TNF and IL-1 to induce this mediator release. OSM has also been demonstrated to induce (with IL-1) collagen and proteoglycan release from cartilage. Furthermore, OSM induces acute phase protein release and production of IL-6 receptor from hepatocytes and may therefore contribute to the systemic effects of rheumatoid inflammation including fatigue. In addition, OSM induces osteoclast differentiation and activity in vitro.

In Vivo evidence

Adenoviral expression of murine OSM (mOSM) in the joints of normal mice results in a severe inflammatory and erosive arthritis. Similarly aggressive disease is seen in knockout mice lacking TNF, IL-1, IL-6 and iNOS following adenoviral mOSM delivery, demonstrating that OSM can mediate all aspects of arthritis pathology. Mouse OSM expression using an adenovirally expressed mOSM vector causes damage to the growth plate typical of Juvenile Idiopathic Arthritis. In an experimental model of collagen induced arthritis, an anti-OSM antibody administered therapeutically to mice prevented all further progression of disease. Similar results were seen when anti-OSM was administered prophylatically to mice with pristane induced arthritis, a relapsing/remitting model reminiscient of the human disease. In monkeys, OSM injected subcutaneously induces an acute phase response and local chronic inflammation. OSM has been demonstrated to induce mononuclear and PMN infiltration and proteoglycan release when injected into goat joints. Transgenic over-expression of mOSM in mouse lymph nodes results in extrathymic T cell maturation, proliferation of memory T cells and failure to deplete autoimmune T cells. Transgenic over-expression of OSM in the pancreas causes extensive fibrosis similar to that seen in advanced RA synovium.

In WO99/48523, we disclose the use of OSM antagonists in the treatment of inflammatory diseases and disorders.

All patent and literature references disclosed within the present specification are expressly and entirely incorporated herein by reference.

Summary of the Invention

The present invention therefore provides a therapeutic antibody or antigen binding fragment thereof which specifically binds to hOSM and interacts with Site II of hOSM.

In another aspect of the present invention there is provided a therapeutic antibody or antigen binding fragment thereof which specifically binds to hOSM and modulates (e.g. inhibits or blocks) the interaction between Site II of hOSM and gp130.

In another aspect, there is provided a therapeutic antibody or antigen binding fragment thereof which specifically binds to hOSM and comprises the following CDRH3: GIYYYGSHYFDY (SEQ.I.D.NO: 3).

In another aspect of the present invention there is provided a therapeutic antibody or antigen binding fragment thereof which specifically binds to hOSM and comprises the following CDRs:
CDRH1: DYNMD (SEQ.I.D.NO: 1)
CDRH2: DINPNNGGTIDNQKFKD (SEQ.I.D.NO: 2)
CDRH3: GIYYYGSHYFDY (SEQ.I.D.NO: 3)
CDRL1: SATSSVSVMH (SEQ.I.D.NO: 4)
CDRL2 DTSKLAS (SEQ.I.D.NO: 5)
CDRL3: QQWSSNPLT (SEQ.I.D.NO: 6)

Throughout this specification, the terms "CDR", "CDRL1", "CDRL2", "CDRL3", "CDRH1", "CDRH2", "CDRH3" follow the Kabat numbering system as set forth in Kabat *et al; Sequences of Proteins of Immunological Interest* NIH, 1987. Therefore the following defines the CDRs according to the invention:

CDR:	Residues
CDRH1:	31-35B

CDRH2: 50-65
CDRH3: 95-102
CDRL1: 24-34
CDRL2: 50-56
CDRL3: 89-97

In another aspect of the invention there is provided a therapeutic antibody or antigen binding fragment thereof comprising a VH domain having the sequence:
EVQLQQSGPELVKPGASVKISCKASGYIFTDYNMDWVKQSHGKKLEWIGDINPNN
GGTIDNQKFKDKATLTVDKSSSTAYMELRSLTSEDTAVYYCARGIYYYGSHYFDY
WGQGTTLTVSS. (SEQ.I.D.NO: 7) and a VL domain having the sequence:
QIVLTQSPAIMSASPGEKVTMTCSATSSVSVMHWFQKKSGTSPKRWIYDTSKLAS
GVPTRFSGSGSGTSYSLTISSMEAEDTATYYCQQWSSNPLTFGSGTKLELK
(SEQ.I.D.NO: 8).

In one aspect of the invention there is provided a humanised therapeutic antibody or antigen binding fragment thereof comprising a VH domain having the sequence set forth in SEQ.I.D.NO: 9:

EVQLVQSGAEVKKPGASVKVSCKASGYIFTDYNMDWVRQAPGQKLEWIGDINPNN
GGTIDNQKFKDRATLTVDKSTSTVYMELSSLRSEDTAVYYCARGIYYYGSHYFDY
WGQGTLVTVSS (SEQ.I.D.NO: 9);

and a VL domain having the sequence set forth in SEQ.I.D.NO:10:

EIVLTQSPSSLSASVGDRVTITCSATSSVSVMHWFQKKPGKAPKRWIYDTSKLAS
GVPSRFSGSGSGTDYTLTISSLQPEDFATYYCQQWSSNPLTFGGGTKVDIK
(SEQ.I.D.NO: 10).

In another aspect of the invention there is provided a humanised therapeutic antibody or antigen binding fragment thereof which modulates (e.g. inhibits or blocks) the interaction between hOSM and gp130.

In another aspect of the invention there is provided an isolated VH domain of an antibody comprising (or consisting essentially of) SEQ.I.D.NO: 7 or 9.

In another aspect of the invention there is provided a therapeutic antibody or antigen binding fragment thereof comprising a VH domain of SEQ.I.D.NO: 7 or 9.

In another aspect of the present invention there is provided a therapeutic antibody or antigen binding fragment thereof which competitively inhibits the binding of the therapeutic antibody comprising the CDRH3 of SEQ I.D.NO: 3 to hOSM.

In another aspect of the invention there is provided a therapeutic antibody comprising a heavy chain of sequence:

EVQLVQSGAEVKKPGASVKVSCKASGYIFTDYNMDWVRQAPGQKLEWIGDINPNNGGTI
DNQKFKDRATLTVDKSTSTVY
MELSSLRSEDTAVYYCARGIYYYGSHYFDYWGQGTLVTVSSASTKGPSVFPLAPSSKST
SGGTAALGCLVKDYFPEPVTV
SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV
EPKSCDKTHTCPPCPAPELLG
GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQ
YNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPP
VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

and a light chain sequence of

EIVLTQSPSSLSASVGDRVTITCSATSSVSVMHWFQKKPGKAPKRWIYDTSKLASGVPS
RFSGSGSGTDYTLTISSLQPE
DFATYYCQQWSSNPLTFGGGTKVDIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY
PREAKVQWKVDNALQSGNSQE
SVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

In another aspect of the invention there is provided a therapeutic antibody or antigen binding fragment thereof which competitively inhibits the binding of the therapeutic antibody comprising CDRs of SEQ.I.D. NO: 1,2,3,4, 5 and 6 to hOSM.

In another aspect of the invention there is provided a method of treating a human patient afflicted with a disease or disorder responsive to modulation of the interaction between hOSM and gp130 which method comprises the step of administering to said patient a therapeutically effective amount of the therapeutic antibody or antigen binding fragment thereof as hereinbefore described.

In another aspect of the present invention there is provided a method of treating a human patient afflicted with an inflammatory disease or disorder which method comprises the step of administering to said patient a therapeutically effective amount of the therapeutic antibody or antigen binding fragment thereof as hereinbefore described.

In another aspect of the present invention there is provided a method of treating a human patient afflicted with an arthritic disease, particularly rheumatoid arthritis, juvenile onset arthritis or osteoarthritis which method comprises the step of administering to said patient a therapeutically effective amount of the therapeutic antibody or antigen binding fragment thereof as hereinbefore described.

In another aspect of the invention there is provided a method of reducing or preventing cartilage degradation in a human patient afflicted with (or suspectible to) such degradation which method comprises the step of

administering a therapeutically effective amount of a therapeutic antibody or antigen binding fragment thereof to said patient as hereinbefore described

In another aspect of the present invention there is provided a method of reducing IL-6 production in a patient afflicted with a disease or disorder responsive to IL-6 reduction which method comprises administering to said patient a therapeutically effective amount of a therapeutic antibody or antigen binding fragment thereof as hereinbefore described.

In another aspect of the invention there is provided a method of treating the extraarticular manifestations of an arthritic disease or disorder which method comprises the step of administering a therapeutically effective amount of a therapeutic antibody or antigen binding fragment thereof as hereinbefore described to said patient.

In another aspect of the present invention there is provided a method of treating a human patient afflicted with a disease of endothelial cell origin which method comprises the steps of administering to said patient a therapeutically effective amount of a therapeutic antibody or antigen binding fragment thereof as hereinbefore described.

Use of the therapeutic antibody or antigen binding fragment thereof as hereinbefore described in the manufacture of a medicament for the treatment of diseases and disorders as hereinbefore described is also provided.

In another aspect of the invention there is provided a process for the manufacture of a therapeutic antibody or antigen binding fragment thereof as hereinbefore described.

In another aspect of the invention there is provided an assay (particularly an ELISA assay) for studying the interaction between OSM (particularly hOSM) and an interacting partner (such as gp130, LIFR, OSMR), which assay comprises the step of providing for said assay, a sample of glycosylated OSM (preferably glycosylated by a invertebrate host cell such as mammalian host cell e.g. CHO glycosylated). Such an assay maybe used to determine the level of hOSM in a human patient (either serum or SF hOSM levels).

Other aspects, objects and advantages of the present invention will be apparent from the description below.

Detailed Description of the Invention

1. Antibody Structures

1.1 Intact Antibodies

Intact antibodies are usually heteromultimeric glycoproteins comprising at least two heavy and two light chains. Aside from IgM, intact antibodies are usually heterotetrameric glycoproteins of approximately 150Kda, composed of two identical light (L) chains and two identical heavy (H) chains. Typically, each light chain is linked to a heavy chain by one covalent disulfide bond while the number of disulfide linkages between the heavy chains of different immunoglobulin isotypes varies. Each heavy

and light chain also has intrachain disulfide bridges. Each heavy chain has at one end a variable domain (VH) followed by a number of constant regions. Each light chain has a variable domain (VL) and a constant region at its other end; the constant region of the light chain is aligned with the first constant region of the heavy chain and the light chain variable domain is aligned with the variable domain of the heavy chain. The light chains of antibodies from any vertebrate species can be assigned to one of two types called Kappa and Lambda based on the amino acid sequence of the constant region. Depending on the amino acid sequence of the constant region of their heavy chains,human antibodies can be assigned to five different classes, IgA, IgD, IgE, IgG and IgM. IgG and IgA can be further subdivided into subclasses, IgG1, IgG2, IgG3 and IgG4; and IgA1 and IgA2. Species variants exist with mouse and rat having IgG2a, IgG2b. The variable domain of the antibody confers binding specificity upon the antibody with certain regions displaying particular variability called complementarity determining regions (CDRs). The more conserved portions of the variable region are called Framework regions (FR). The variable domains of intact heavy and light chains each comprise four FR connected by three CDRs. The CDRs in each chain are held together in close proximity by the FR regions and with the CDRs from the other chain contribute to the formation of the antigen binding site of antibodies. The constant regions are not directly involved in the binding of the antibody to the antigen but exhibit various effector functions such as participation in antibody dependent cell-mediated cytotoxicity (ADCC), phagocytosis via binding to Fcγ receptor, half-life/clearance rate via neonatal Fc receptor (FcRn) and complement dependent cytotoxicity via the C1q component of the complement cascade.

In one embodiment therefore we provide an intact therapeutic antibody that specifically binds to hOSM, which antibody modulates the interaction between hOSM and gp130. The antibody may specifically bind to Site II

of hOSM and inhibit or block the interaction between hOSM and its corresponding residues on gp130 involved in OSM interaction. The ELISA protocol of section 7 of the examples may be used to determine whether any particular antibody or antigen binding fragment thereof modulates the interaction between hOSM and gp130. The therapeutic intact antibody may comprise a constant region of any isotype or subclass thereof described *supra*. In one embodiment, the antibody is of the IgG isotype, particularly IgG1 or IgG4. The antibody may be rat, mouse, rabbit, primate or human. In one typical embodiment, the antibody maybe primate (such as cynomolgus, Old World monkey or Great Ape, see e.g. WO99/55369, WO93/02108) or human.

In another embodiment there is provided an intact therapeutic antibody comprising a CDRH3 of SEQ.I.D.NO: 3. In another embodiment there is provided an intact therapeutic antibody comprising a variable region having CDRs of SEQ.I.D.NO: 1,2,3,4,5 and 6 together with a constant region of IgG isotype, particularly IgG1 or IgG4.

In another embodiment, there is provided a murine intact therapeutic antibody or antigen binding fragment thereof comprising a VH domain having the sequence of SEQ.I.D.NO: 7 and a VL domain of the sequence of SEQ.I.D.NO: 8.

1.1.2 Human antibodies

Human antibodies may be produced by a number of methods known to those of skill in the art. Human antibodies can be made by the hybridoma method using human myeloma or mouse-human heteromyeloma cells lines see Kozbor J.Immunol 133,3001, (1984) and Brodeur, Monoclonal Antibody Production Techniques and Applications, pp51-63 (Marcel

Dekker Inc, 1987). Alternative methods include the use of phage libraries or transgenic mice both of which utilize human V region repertories (see Winter G, (1994), Annu.Rev.Immunol 12,433-455, Green LL (1999), J.Immunol.methods 231,11-23).
Several strains of transgenic mice are now available wherein their mouse immunoglobulin loci has been replaced with human immunoglobulin gene segments (see Tomizuka K, (2000) PNAS 97,722-727; Fishwild D.M (1996) Nature Biotechnol. 14,845-851, Mendez MJ, 1997, Nature Genetics, 15,146-156). Upon antigen challenge such mice are capable of producing a repertoire of human antibodies from which antibodies of interest can be selected. Of particular note is the Trimera™ system (see Eren R *et al*, (1998) Immunology 93:154-161) where human lymphocytes are transplanted into irradiated mice, the Selected Lymphocyte Antibody System (SLAM, see Babcook *et al*, PNAS (1996) 93:7843-7848) where human (or other species) lymphocytes are effectively put through a massive pooled *in vitro* antibody generation procedure followed by deconvulated, limiting dilution and selection procedure and the Xenomouse II™ (Abgenix Inc). An alternative approach is available from Morphotek Inc using the Morphodoma™ technology.

Phage display technology can be used to produce human antibodies (and fragments thereof), see McCafferty; Nature, 348, 552-553 (1990) and Griffiths AD *et al* (1994) EMBO 13:3245-3260. According to this technique antibody V domain genes are cloned in frame into either a major or minor coat of protein gene of a filamentous bacteriophage such as M13 or fd and displayed (usually with the aid of a helper phage) as functional antibody fragments on the surface of the phage particle. Selections based on the functional properties of the antibody result in selection of the gene encoding the antibody exhibiting those properties. The phage display technique can be used to select antigen specific

antibodies from libraries made from human B cells taken from individuals afflicted with a disease or disorder described above or alternatively from unimmunized human donors (see Marks; J.Mol.Bio. 222,581-597, 1991). Where an intact human antibody is desired comprising a Fc region it is necessary to reclone the phage displayed derived fragment into a mammalian expression vectors comprising the desired constant regions and establishing stable expressing cell lines.
The technique of affinity maturation (Marks; Bio/technol 10,779-783 (1992)) may be used to improve binding affinity wherein the affinity of the primary human antibody is improved by sequentially replacing the H and L chain V regions with naturally occurring variants and selecting on the basis of improved binding affinities. Variants of this technique such as "epitope imprinting" are now also available see WO 93/06213. See also Waterhouse; Nucl.Acids Res 21, 2265-2266 (1993).

Thus in another embodiment there is provided a human intact therapeutic antibody or antigen binding fragment thereof which specifically binds to hOSM and modulates (e.g. inhibits or blocks) the interaction between hOSM and gp130. In another embodiment there is provided a human intact therapeutic antibody or antigen binding fragment thereof which specifically binds to Site II of hOSM and modulates (e.g. inhibits or blocks) the interaction between hOSM and gp130.

In another aspect there is provided a human intact therapeutic antibody or antigen binding fragment thereof comprising a CDRH3 of SEQ.I.D.NO: 3 which specifically binds hOSM and modulates (e.g. inhibits or blocks) the interaction between hOSM and gp130. In another aspect there is provided a human intact therapeutic antibody or antigen binding fragment thereof comprising a variable region having CDRs of SEQ.I.D.NO: 1,2,3,

4, 5 and 6 as defined *supra*. The human intact thereapeutic antibody may comprise a IgG isotype, e.g. IgG1 or IgG4.

1.2 Chimaeric and Humanised Antibodies

The use of intact non-human antibodies in the treatment of human diseases or disorders carries with it the now well established problems of immunogenicity, that is the immune system of the patient may recognise the non-human intact antibody as non-self and mount a neutralising response. Various techniques have been developed over the years to overcome these problems and generally involve reducing the composition of non-human amino acid sequences in the intact antibody whilst retaining the relative ease in obtaining non-human antibodies from an immunised animal e.g. mouse, rat or rabbit. Broadly two approaches have been used to achieve this. The first are chimaeric antibodies, which generally comprise a non-human (e.g. rodent such as mouse) variable domain fused to a human constant region. Because the antigen-binding site of an antibody is localised within the variable regions the chimaeric antibody retains its binding affinity for the antigen but acquires the effector functions of the human constant region and are therefore able to perform effector functions such as described *supra*. Chimaeric antibodies are typically produced using recombinant DNA methods. DNA encoding the antibodies (e.g. cDNA) is isolated and sequenced using conventional procedures (e.g. by using oligonucleotide probes that are capable of binding specifically to genes encoding the H and L chains of the antibody of the invention, e.g. DNA encoding SEQ.I.D.NO 1,2,3,4,5 and 6 described *supra*). Hybridoma cells serve as a typical source of such DNA. Once isolated, the DNA is placed into expression vectors which are then

transfected into host cells such as *E.Coli*, COS cells, CHO cells or myeloma cells that do not otherwise produce immunoglobulin protein to obtain synthesis of the antibody. The DNA may be modified by substituting the coding sequence for human L and H chains for the corresponding non-human (e.g. murine) H and L constant regions see e.g. Morrison; PNAS 81, 6851 (1984).

The second approach involves the generation of humanised antibodies wherein the non-human content of the antibody is reduced by humanizing the variable regions. Two techniques for humanisation have gained popularity. The first is humanisation by CDR grafting. CDRs build loops close to the antibody's N-terminus where they form a surface mounted in a scaffold provided by the framework regions. Antigen-binding specificity of the antibody is mainly defined by the topography and by the chemical characteristics of its CDR surface. These features are in turn determined by the conformation of the individual CDRs, by the relative disposition of the CDRs, and by the nature and disposition of the side chains of the residues comprising the CDRs. A large decrease in immunogenicity can be achieved by grafting only the CDRs of a non-human (e.g. murine) antibodies ("donor" antibodies) onto human framework ("acceptor framework") and constant regions (see Jones *et al* (1986) Nature 321,522-525 and Verhoeyen M *et al* (1988) Science 239,1534-1536). However, CDR grafting *per se* may not result in the complete retention of antigen-binding properties and it is frequently found that some framework residues of the donor antibody need to be preserved in the humanised molecule if significant antigen-binding affinity is to be recovered (see Queen C *et al* (1989) PNAS 86, 10,029-10,033, Co, M *et al* (1991) Nature 351, 501-502). In this case, human V regions showing the greatest sequence homology to the non-human donor antibody are chosen from a database in order to provide the human framework (FR). The selection of human FRs can be

made either from human consensus or individual human antibodies. Where necessary key residues from the donor antibody are substituted into the human acceptor framework to preserve CDR conformations. Computer modelling of the antibody maybe used to help identify such structurally important residues, see WO99/48523.

Alternatively, humanisation maybe achieved by a process of "veneering". A statistical analysis of unique human and murine immunoglobulin heavy and light chain variable regions revealed that the precise patterns of exposed residues are different in human and murine antibodies, and most individual surface positions have a strong preference for a small number of different residues (see Padlan E.A. *et al*; (1991) Mol.Immunol.28, 489-498 and Pedersen J.T. *et al* (1994) J.Mol.Biol. 235; 959-973). Therefore it is possible to reduce the immunogenicity of a non-human Fv by replacing exposed residues in its framework regions that differ from those usually found in human antibodies. Because protein antigenicity can be correlated with surface accessibility, replacement of the surface residues may be sufficient to render the mouse variable region "invisible" to the human immune system (see also Mark G.E. *et al* (1994) in *Handbook of Experimental Pharmacology vol.113: The pharmacology of monoclonal Antib*odies, Springer-Verlag, pp105-134). This procedure of humanisation is referred to as "veneering" because only the surface of the antibody is altered, the supporting residues remain undisturbed.

Thus another embodiment of the invention there is provided a chimaeric therapeutic antibody comprising a non-human (e.g. rodent) variable domain fused to a human constant region (which maybe of a IgG isotype e.g. IgG1) which specifically binds to hOSM and modulates the interaction between Site II of hOSM and gp130.

In another embodiment there is provided a chimaeric therapeutic antibody comprising a non-human (e.g. rodent) variable region and a human constant region (which maybe of an IgG isotype e.g. IgG1) which specifically binds to hOSM, which antibody further comprises a CDRH3 of SEQ.I.D.NO3. Such antibodies may further comprise a human constant region of the IgG isotype, e.g. IgG1

In another embodiment there is chimaeric therapeutic antibody comprising a non-human (e.g. rodent) variable region and a human constant region (which maybe of a IgG isotype e.g. IgG1) which specifically binds to hOSM having the CDRs of SEQ.I.D.NO: 1,2,3,4,5 and 6.

In another embodiment there is provided a humanised therapeutic antibody or antigen binding fragment thereof which specifically binds to hOSM and modulates (e.g. inhibits or blocks) the interaction between Site II of hOSM and gp130.
In another embodiment there is provided a humanised therapeutic antibody or antigen binding fragment thereof which specifically binds to hOSM and comprises a CDRH3 of SEQ.I.D.NO: 3. Such antibodies may comprise a human constant region of the IgG isotype, e.g. IgG1.

In another embodiment there is provided a humanised therapeutic antibody or antigen binding fragment thereof which specifically binds to hOSM and comprises CDRs of SEQ.I.D.NO1, 2,3,4,5 and 6. Such antibodies may comprise a human constant region of the IgG isotype, e.g. IgG1.

In another embodiment there is provided a humanised therapeutic antibody or antigen binding fragment thereof which specifically binds to

hOSM and comprises the following heavy chain of SEQ.I.D.NO: 11 and a light chain of SEQ.I.D.NO: 12.

In another embodiment there is provided a humanised therapeutic antibody or antigen binding fragment thereof which specifically binds to hOSM wherein said antibody or fragment thereof comprises CDRH3 (SEQ.I.D.NO: 3) optionally further comprising CDRs of SEQ.I.D.NO: 1,2,4,5 and 6 wherein the residues at positions 28,44,48,67, 69, 71,73 of the human acceptor heavy chain framework region and positions 36,38,46,47,71 of the human acceptor light chain framework are substituted by the corresponding residues found in the donor antibody framework from which CDRH3 is derived.

It will be apparent to those skilled in the art that the term "derived" is intended to define not only the source in the sense of it being the *physical* origin for the material but also to define material which is structually identical to the material but which does not originate from the reference source. Thus "residues found in the donor antibody from which CDRH3 is derived" need not necessarily have been purified from the donor antibody.

In another embodiment there is provided a humanised therapeutic antibody or antigen binding fragment thereof which specifically binds to hOSM wherein said antibody or fragment thereof comprises CDRH3 (SEQ.I.D.NO: 3) optionally further comprising CDRs of SEQ.I.D.NO: 1,2,4,5 and 6 wherein the human heavy chain framework comprises-one or more (e.g. all) of the following residues (or a conservative substitute thereof):

Position	**Residue**
28	I

48	I
44	K
67	A
69	L
71	V
73	K

and the human light chain comprises one or more (e.g. all) of the following residues (or conservative substitute thereof);

Position	**Residue**
36	F
38	K
46	R
47	W
71	Y

In another embodiment there is provided a humanised therapeutic antibody or antigen binding fragment thereof which specifically binds to hOSM wherein said antibody or fragment thereof comprises CDRs of SEQ.I.D.NO: 1,2,3,4,5 and 6 wherein the human heavy chain framework comprises one or more (e.g. all) of the following residues (or a conservative substitute thereof):

Position	**Residue**
28	I
48	I
44	K
67	A
69	L
71	V
73	K

and the human light chain comprises one or more (e.g.all) of the following residues (or conservative substitute thereof);

Position	**Residue**
36	F
38	K
46	R
47	W
71	Y

It is well recognised in the art that certain amino acid substitutions are regarded as being "conservative". Amino acids are divided into groups based on common side-chain properties and substitutions within groups that maintain all or substantially all of the binding affinity of the antibody of the invention or antigen binding fragment thereof are regarded as conservative substitutions, see the following table:

Side chain	Members
hydrophobic	met, ala,val,leu,ile
neutral hydrophilic	cys, ser, thr
acidic	asp, glu
basic	asn, gln, his, lys, arg
residues that influence chain orientation	gly, pro
Aromatic	trp, tyr, phe

1.3 Bispecific antibodies

A bispecific antibody is an antibody having binding specificities for at least two different epitopes. Methods of making such antibodies are known in the art. Traditionally, the recombinant production of bispecific antibodies is based on the coexpression of two immunoglobulin H chain-L chain

pairs, where the two H chains have different binding specificities see Millstein *et al*, Nature 305 537-539 (1983), WO93/08829 and Traunecker *et al* EMBO, 10, 1991, 3655-3659. Because of the random assortment of H and L chains, a potential mixture of ten different antibody structures are produced of which only one has the desired binding specificity. An alternative approach involves fusing the variable domains with the desired binding specificities to heavy chain constant region comprising at least part of the hinge region, CH2 and CH3 regions. It is preferred to have the CH1 region containing the site necessary for light chain binding present in at least one of the fusions. DNA encoding these fusions, and if desired the L chain are inserted into separate expression vectors and are then cotransfected into a suitable host organism. It is possible though to insert the coding sequences for two or all three chains into one expression vector. In one preferred approach, the bispecific antibody is composed of an H chain with a first binding specificity in one arm and a H-L chain pair, providing a second binding specificity in the other arm, see WO94/04690. Also see Suresh *et al* Methods in Enzymology 121, 210, 1986.

In one embodiment of the invention there is provided a bispecific therapeutic antibody wherein at least one binding specificity of said antibody is for hOSM, wherein said antibody modulates (e.g. inhibits or blocks) the interaction between Site II of hOSM and gp130. Such antibodies may further comprise a human constant region of the IgG isotype, e.g. IgG1

In one embodiment of the invention there is provided a bispecific therapeutic antibody wherein at least one binding specificity of said antibody is for hOSM, wherein said antibody comprises a CDRH3 of SEQ.I.D.NO: 3. Such antibodies may further comprise a human constant region of the IgG isotype, e.g. IgG1.

In one embodiment of the invention there is provided a bispecific therapeutic antibody wherein at least one binding specificity of said antibody is for hOSM, wherein said antibody comprises at least CDRs of SEQ.I.D.NO: 1,2,3,4,5 and 6. Such antibodies may further comprise a human constant region of the IgG isotype, e.g. IgG1.

1.4 Antibody Fragments

In certain embodiments of the invention there is provided therapeutic antibody fragments which modulate the interaction between OSM (particularly hOSM) and gp130. Such fragments may be functional antigen binding fragments of intact and/or humanised antibodies such as Fab, Fab', $F(ab')_2$, Fv, ScFv fragments of the antibodies described *supra*. Traditionally such fragments are produced by the proteolytic digestion of intact antibodies by e.g. papain digestion (see for example, WO 94/29348) but may be produced directly from recombinantly transformed host cells. For the production of ScFv, see Bird *et al*;(1988) Science, 242, 423-426. In addition, antibody fragments may be produced using a variety of engineering techniques as described below.

Fv fragments appear to have lower interaction energy of their two chains than Fab fragments. To stablise the association of the VH and VL domains, they have been linked with peptides (Bird *et al*, (1988) Science 242, 423-426, Huston *et al*, PNAS, 85,5879-5883), disulphide bridges (Glockshuber *et al*, (1990) Biochemistry, 29, 1362-1367) and "knob in hole" mutations (Zhu *et al* (1997), Protein Sci., 6, 781-788). ScFv fragments can be produced by methods well known to those skilled in the art see Whitlow *et al* (1991) Methods companion Methods Enzymol, 2, 97-

105 and Huston *et al* (1993) Int.Rev.Immunol 10, 195-217. ScFv may be produced in bacterial cells such as *E.Coli* but are more preferably produced in eukaryotic cells. One disadvantage of ScFv is the monovalency of the product, which precludes an increased avidity due to polyvalent binding, and their short half-life. Attempts to overcome these problems include bivalent $(ScFv')_2$ produced from ScFV containing an additional C terminal cysteine by chemical coupling (Adams *et al* (1993) Can.Res 53, 4026-4034 and McCartney *et al* (1995) Protein Eng. 8, 301-314) or by spontaneous site-specific dimerization of ScFv containing an unpaired C terminal cysteine residue (see Kipriyanov *et al* (1995) Cell. Biophys 26, 187-204). Alternatively, ScFv can be forced to form multimers by shortening the peptide linker to 3 to 12 residues to form "diabodies", see Holliger *et al* PNAS (1993), 90, 6444-6448. Reducing the linker still further can result in ScFV trimers ("triabodies", see Kortt *et al* (1997) Protein Eng, 10, 423-433) and tetramers ("tetrabodies", see Le Gall *et al* (1999) FEBS Lett, 453, 164-168). Construction of bivalent ScFV molecules can also be achieved by genetic fusion with protein dimerizing motifs to form "miniantibodies" (see Pack *et al* (1992) Biochemistry 31, 1579-1584) and "minibodies" (see Hu *et al* (1996), Cancer Res. 56, 3055-3061). ScFv-Sc-Fv tandems ((ScFV)2) may also be produced by linking two ScFv units by a third peptide linker, see Kurucz *et al* (1995) J.Immol.154, 4576-4582. Bispecific diabodies can be produced through the noncovalent association of two single chain fusion products consisting of VH domain from one antibody connected by a short linker to the VL domain of another antibody, see Kipriyanov *et al* (1998), Int.J.Can 77,763-772. The stability of such bispecific diabodies can be enhanced by the introduction of disulphide bridges or "knob in hole" mutations as described *supra* or by the formation of single chain diabodies (ScDb) wherein two hybrid ScFv fragments are connected through a peptide linker see Kontermann *et al* (1999) J.Immunol.Methods 226 179-188. Tetravalent

bispecific molecules are available by e.g. fusing a ScFv fragment to the CH3 domain of an IgG molecule or to a Fab fragment through the hinge region see Coloma *et al* (1997) Nature Biotechnol. 15, 159-163. Alternatively, tetravalent bispecific molecules have been created by the fusion of bispecific single chain diabodies (see Alt *et al*, (1999) FEBS Lett 454, 90-94. Smaller tetravalent bispecific molecules can also be formed by the dimerization of either ScFv-ScFv tandems with a linker containing a helix-loop-helix motif (DiBi miniantibodies, see Muller *et al* (1998) FEBS Lett 432,45-49) or a single chain molecule comprising four antibody variable domains (VH and VL) in an orientation preventing intramolecular pairing (tandem diabody, see Kipriyanov *et al*, (1999) J.Mol.Biol. 293, 41-56). Bispecific F(ab')2 fragments can be created by chemical coupling of Fab' fragments or by heterodimerization through leucine zippers (see Shalaby *et al*, (1992) J.Exp.Med. 175, 217-225 and Kostelny *et al* (1992), J.Immunol. 148, 1547-1553).

In one embodiment there is provided a therapeutic antibody fragment (e.g. ScFv, Fab, Fab', $F(ab')_2$) or an engineered antibody fragment as described *supra*) that specifically binds to hOSM and modulates (e.g. , inhibits or blocks) the interaction between Site II of hOSM and gp130. The therapeutic antibody fragment typically comprises a CDRH3 having the sequence of SEQ.I.D.NO: 3 optionally together with CDRs having the sequence set forth in SEQ.I.D.NO: 1,2,4,5 and 6.

1.5 Heteroconjugate antibodies

Heteroconjugate antibodies also form an embodiment of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies formed using any convenient cross-linking methods. See US 4,676,980.

1.6 Other Modifications.

The interaction between the Fc region of an antibody and various Fc receptors (FcγR) is believed to mediate the effector functions of the antibody which include antibody-dependent cellular cytotoxicity (ADCC), fixation of complement, phagocytosis and half-life/clearance of the antibody. Various modifications to the Fc region of antibodies of the invention may be carried out depending on the desired property. For example, specific mutations in the Fc region to render an otherwise lytic antibody, non-lytic is detailed in EP 0629 240B1 and EP 0307 434B2 or one may incorporate a salvage receptor binding epitope into the antibody to increase serum half life see US 5,739,277. There are five currently recognised human Fcγ receptors, FcγR (I), FcγRIIa, FcγRIIb, FcγRIIIa and neonatal FcRn. Shields *et al*, (2001) J.Biol.Chem 276, 6591-6604 demonstrated that a common set of IgG1 residues is involved in binding all FcγRs, while FcγRII and FcγRIII utilize distinct sites outside of this common set. One group of IgG1 residues reduced binding to all FcγRs when altered to alanine: Pro-238, Asp-265, Asp-270, Asn-297 and Pro-239. All are in the IgG CH2 domain and clustered near the hinge joining CH1 and CH2. While FcγRI utilizes only the common set of IgG1 residues for binding, FcγRII and FcγRIII interact with distinct residues in addition to the common set. Alteration of some residues reduced binding only to FcγRII (e.g. Arg-292) or FcγRIII (e.g. Glu-293). Some variants showed improved binding to FcγRII or FcγRIII but did not affect binding to the other receptor (e.g. Ser-267Ala improved binding to FcγRII but binding to FcγRIII was unaffected). Other variants exhibited improved binding to FcγRII or FcγRIII with reduction in binding to the other receptor (e.g. Ser-298Ala improved binding to FcγRIII and reduced binding to FcγRII). For FcγRIIIa,

the best binding IgG1 variants had combined alanine substitutions at Ser-298, Glu-333 and Lys-334. The neonatal FcRn receptor is believed to be involved in both antibody clearance and the transcytosis across tissues (see Junghans R.P (1997) Immunol.Res 16. 29-57 and Ghetie *et al* (2000) Annu.Rev.Immunol. 18, 739-766). Human IgG1 residues determined to interact directly with human FcRn includes Ile253, Ser254, Lys288, Thr307, Gln311, Asn434 and His435. Switches at these positions may enable increased serum half-life of the antibodies of the invention.

Other modifications include glycosylation variants of the antibodies of the invention. Glycosylation of antibodies at conserved positions in their constant regions is known to have a profound effect on antibody function, particularly effector functioning such as those described above, see for example, Boyd *et al* (1996), Mol.Immunol. 32,1311-1318. Glycosylation variants of the therapeutic antibodies or antigen binding fragments thereof of the present invention wherein one or more carbonhydrate moiety is added, substituted, deleted or modified are contemplated. Introduction of an asparagine-X-serine or asparagine-X-threonine motif creates a potential site for enzymatic attachment of carbonhydrate moieties and may therefore be used to manipulate the glycosylation of an antibody. In Raju *et al* (2001) Biochemistry 40, 8868-8876 the terminal sialyation of a TNFR-IgG immunoadhesin was increased through a process of regalactosylation and/or resialylation using beta-1, 4-galactosyltransferace and/or alpha, 2,3 sialyltransferase. Increasing the terminal sialylation is believed to increase the half-life of the immunoglobulin. Antibodies, in common with most glycoproteins, are typically produced as a mixture of glycoforms. This mixture is particularly apparent when antibodies are produced in eukaryotic, particularly mammalian cells. A variety of methods have been developed to manufacture defined glycoforms, see Zhang *et al* Science (2004), 303, 371, Sears *et al*, Science, (2001) 291, 2344, Wacker *et al*

(2002) Science, 298 1790, Davis *et al* (2002) Chem.Rev. 102, 579, Hang *et al* (2001) Acc.Chem.Res 34, 727. Thus the invention contemplates a plurality of therapeutic (monoclonal) antibodies (which maybe of the IgG isotype, e.g. IgG1) as hereinbefore described comprising a defined number (e.g. 7 or less, for example 5 or less such as two or a single) glycoform(s) of said antibodies or antigen binding fragments thereof.

Further embodiments of the invention include therapeutic antibodies of the invention or antigen binding fragments thereof coupled to a non-proteinaeous polymer such as polyethylene glycol (PEG), polypropylene glycol or polyoxyalkylene. Conjugation of proteins to PEG is an established technique for increasing half-life of proteins, as well as reducing antigenicity and immunogenicity of proteins. The use of PEGylation with different molecular weights and styles (linear or branched) has been investigated with intact antibodies as well as Fab' fragments, see Koumenis I.L. *et al* (2000) Int.J.Pharmaceut. 198:83-95.

Delivery of therapeutic proteins to the brain has been hampered by the presence of the blood brain barrier (BBB). Where it is desired to deliver an antibody of the invention or antibody fragment of the invention to the brain various strategies have been proposed to enhance such delivery where needed.

In order to obtain required nutrients and factors from the blood, the BBB posseses some specific receptors, which transport compounds from the circulating blood to the brain. Studies have indicated that some compounds like insulin (see Duffy KR *et al* (1989) Brain Res. 420:32-38), transferin (see Fishman JB *et al* (1987) J.Neurosci 18:299-304) and insulin like growth factors 1 and 2 (see Pardridge WM (1986) Endocrine Rev.7:314-330 and Duffy KR *et al* (1986) Metabolism 37:136-140)

traverse the BBB via receptor-mediated transcytosis. Receptors for these molecules thus provide a potential means for antibodies of the invention and/or antibody fragments of the invention to access the brain using so – called "vectored" antibodies (see Pardridge WM (1999) Advanced Drug Delivery Review 36:299-321). For example, an antibody to transferrin receptor has been shown to be dynamically transported into the brain parenchyma (see Friden PM *et al* (1991) PNAS 88:4771-4775 and Friden PM *et al* (1993) Science 259:373-377). Thus one potential approach is to produce a bispecific antibody or bispecific fragment such as described *supra* wherein a first specificity is towards Site II of hOSM (e.g. the first specificity comprises CDRH3 of SEQ.I.D.NO: 3 optionally together with CDRs of SEQ.I.D.NO: 1,2,4,5 and 6) and a second specificity towards a transport receptor located at the BBB e.g. a second specificity towards the transferrin transport receptor.

2. Competing antibodies

The present invention also contemplates antibodies and antigen binding fragments of antibodies which specifically bind OSM, particularly hOSM and competitively inhibit, at equimolar concentrations, the binding to OSM, particularly hOSM of the therapeutic antibody of the invention or antigen binding fragment thereof comprising a CDRH3 of SEQ.I.D.NO: 3 and/or a therapeutic antibody or antigen binding fragment thereof comprising CDRs of SEQ.I.D.NO: 1,2,3,4,5 and 6. The competing antibody or antibody fragment displays,at equimolar concentrations, at least 25% inhibition, typically 35% or greater, more typically at least 50% inhibition.

Thus in one embodiment of the invention there is provided a method of screening a candidate antibody or antibody fragment to determine whether the candidate antibody or antibody fragment is a competing antibody as herein described which method comprises the steps of;

(a) incubating the candidate antibody or antibody fragment with a therapeutic antibody comprising CDRH3 of SEQ.I.D.NO: 3, optionally further comprising CDRs of SEQ.I.D.NO: 1,2,4,5 and 6 or antigen binding fragment thereof;
(b) determining whether the candidate antibody or antibody fragment thereof of step (a) competitively inhibits the binding of the therapeutic antibody or antigen binding fragment thereof to OSM and in particular hOSM.

Thus there is also provided a competing antibody or antigen binding fragment thereof which competitively inhibits the binding of a therapeutic antibody or antigen binding fragment thereof which therapeutic antibody or antigen binding fragment thereof comprises CDR having the sequences set forth in SEQ.I.D.NO: 1,2,3,4,5 and 6.

A competing antibody or antigen binding fragment thereof maybe of any of the above antibody structures. For example, the competing antibody may be a primate or human intact antibody or a humanised antibody preferably of an IgG isotype e.g. IgG1 or IgG4. Competing antibody fragments maybe Fab, Fab', $F(ab')_2$, ScFv and the like. A competing antibody may be produced according to the methods disclosed within this present specification.

A typical protocol for the screening method described *supra*, is set forth in the examples below.

3. Production Methods

Antibodies of the invention maybe produced as a polyclonal population but are more preferably produced as a monoclonal population (that is as a substantially homogenous population of identical antibodies directed against a specific antigenic binding site). Antibodies of the present invention may be produced in transgenic organisms such as goats (see Pollock *et al* (1999), J.Immunol.Methods 231:147-157), chickens (see Morrow KJJ (2000) Genet.Eng.News 20:1-55, mice (see Pollock *et al*) or plants (see Doran PM, (2000) Curr.Opinion Biotechnol. 11, 199-204, Ma JK-C (1998), Nat.Med. 4; 601-606, Baez J *et al*, BioPharm (2000) 13: 50-54, Stoger E *et al*; (2000) Plant Mol.Biol. 42:583-590). Antibodies may also be produced by chemical synthesis. However, antibodies of the invention are typically produced using recombinant cell culturing technology well known to those skilled in the art. A polynucleotide encoding the antibody is isolated and inserted into a replicable vector such as a plasmid for further cloning (amplification) or expression. One useful expression system is a glutamate synthetase system (such as sold by Lonza Biologics), particularly where the host cell is CHO or NS0 (see below). Polynucleotide encoding the antibody is readily isolated and sequenced using conventional procedures (e.g. oligonucleotide probes). Vectors that may be used include plasmid, virus, phage, transposons, minichromsomes of which plasmids are a typical embodiment. Generally such vectors further include a signal sequence, origin of replication, one or more marker genes, an enhancer element, a promoter and transcription termination sequences operably linked to the light and/or heavy chain polynucleotide so as to facilitate expression. Polynucleotide encoding the light and heavy chains may be inserted into separate vectors and transfected into the same host cell or, if desired both the heavy chain and light chain can be inserted into the same vector for transfection into the

host cell. Thus according to one aspect of the present invention there is provided a process of constructing a vector encoding the light and/or heavy chains of a therapeutic antibody or antigen binding fragment thereof of the invention, which method comprises inserting into a vector, a polynucleotide encoding either a light chain and/or heavy chain of a therapeutic antibody of the invention.

In other aspect of the invention there is provided a polynucleotide encoding a murine VH domain having the sequence set forth as SEQ.I.D.NO:15:

GAGGTCCAGCTGCAACAGTCTGGACCTGAGCTGGTGAAGCCTGGGGCTTCAGTGAAGAT
ATCCTGCAAGGCCTCTGGATA
CATATTCACTGACTACAACATGGACTGGGTGAAGCAGAGCCATGGAAAGAAACTTGAGT
GGATTGGAGATATTAATCCTA
ATAATGGTGGTACTATCGACAACCAGAAGTTCAAGGACAAGGCCACATTGACTGTAGAC
AAGTCCTCCAGCACAGCCTAC
ATGGAGCTCCGCAGCCTGACATCTGAGGACACTGCAGTCTATTACTGTGCAAGAGGGAT
TTATTACTACGGTAGTCACTA
CTTTGACTATTGGGGCCAAGGCACCACTCTCACAGTCTCCTCA

In another aspect of the invention there is provided polynucleotide encoding a murine VL domain having the sequence set forth as SEQ.I.D.NO: 16:

CAAATTGTTCTCACCCAGTCTCCAGCAATCATGTCTGCATCTCCAGGGGAGAAGGTCAC
CATGACCTGCAGTGCCACCTC
AAGTGTAAGTGTCATGCACTGGTTCCAGAAGAAGTCAGGTACCTCCCCCAAAAGATGGA
TTTATGACACATCCAAACTGG
CTTCTGGAGTCCCTACTCGCTTCAGTGGCAGTGGGTCTGGGACCTCTTACTCTCTCACA
ATCAGTAGCATGGAGGCTGAA
GATACTGCCACTTATTACTGCCAGCAGTGGAGTAGTAACCCACTCACGTTCGGTTCTGG
GACCAAGCTGGAGCTGAAA

(SEQ.I.D.NO: 16)

In another embodiment there is provided a polynucletotide encoding a humanised VH domain having the sequence set forth as SEQ.I.D.NO: 17:

GAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
TTCCTGCAAGGCATCTGGATA
CATATTCACCGACTACAACATGGACTGGGTGCGACAGGCCCCTGGACAAAAACTTGAGT
GGATTGGAGATATTAATCCTA

ATAATGGTGGTACTATCGACAACCAGAAGTTCAAGGACAGAGCCACCTTGACCGTAGAC
AAGTCCACGAGCACAGTCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGGAT
TTATTACTACGGTAGTCACTA
CTTTGACTATTGGGGCCAGGGAACACTAGTCC
(SEQ.I.D.NO: 17)

In another embodiment there is provided a polynucleotide encoding a humanised VL chain having the sequence set forth as SEQ.I.D.NO: 18:

GAAATTGTGTTGACGCAGTCTCCATCCTCCCTGTCTGCATCTGTTGGAGACAGAGTCAC
CATCACTTGCAGTGCCACCTC
AAGTGTAAGTGTCATGCACTGGTTCCAGAAGAAACCAGGGAAAGCCCCTAAGAGATGGA
TCTATGACACATCCAAACTGG
CTTCTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTACACTCTCACC
ATCAGCAGTCTGCAACCTGAA

GATTTTGCAACTTATTACTGCCAGCAGTGGAGTAGTAACCCACTCACGTTCGGCGGAGG
GACCAAAGTGGATATCAAA

In another embodiment there is provided a polynucleotide encoding a humanised heavy chain having the sequence set forth:

GAGGTCCAGCTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
TTCCTGCAAGGCATCTGGATA
CATATTCACCGACTACAACATGGACTGGGTGCGACAGGCCCCTGGACAAAAACTTGAGT
GGATTGGAGATATTAATCCTA
ATAATGGTGGTACTATCGACAACCAGAAGTTCAAGGACAGAGCCACCTTGACCGTAGAC
AAGTCCACGAGCACAGTCTAC
ATGGAGCTGAGCAGCCTGAGATCTGAGGACACGGCCGTGTATTACTGTGCGAGAGGGAT
TTATTACTACGGTAGTCACTA
CTTTGACTATTGGGGCCAGGGAACACTAGTCACAGTCTCCTCAGCCTCCACCAAGGGCC
CATCGGTCTTCCCCCTGGCAC
CcTCCtCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTAC
TTCCCCGAACCGGTGACGGTG
TCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTC
CTCAGGACTCTACTCCCTCAG
CAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGA
ATCACAAGCCCAGCAACACCA
AGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGC
CCAGCACCTGAACTCCTGGGG
GGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGAC
CCCTGAGGTCACATGCGTGGT
GGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGG
AGGTGCATAATGCCAAGACAA
AGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTG
CACCAGGACTGGCTGAATGGC

AAGGAGTACAAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCAtCGAGAAAACCaT
CTCCAAAGCCAAAGGGCAGCC
CCGAGAACCACAGGTGTACACCCTGCCCCCaTCCCGGGATGAGCTGACCAAGaACCAGG
TCAGCCTGACCTGCCTGGTCA
AAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAAC
AACTACAAGACCACGCCTCCC
GTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAG
GTGGCAGCAGGGGAACGTcTT

CTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCC

TGTCTCCGGGTAAAT

In another embodiment there is provided a polynucleotide encoding a humanised light chain having the sequence set forth:

GAAATTGTGTTGACGCAGTCTCCATCCTCCCTGTCTGCATCTGTTGGAGACAGAGTCAC
CATCACTTGCAGTGCCACCTC
AAGTGTAAGTGTCATGCACTGGTTCCAGAAGAAACCAGGGAAAGCCCCTAAGAGATGGA
TCTATGACACATCCAAACTGG
CTTCTGGGGTCCCATCAAGGTTCAGTGGCAGTGGATCTGGGACAGATTACACTCTCACC
ATCAGCAGTCTGCAACCTGAA
GATTTTGCAACTTATTACTGCCAGCAGTGGAGTAGTAACCCACTCACGTTCGGCGGAGG
GACCAAAGTGGATATCAAACG
TACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTG
GAACTGCCTCTGTTGTGTGCC
TGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGACAACGCCCTC
CAATCGGGTAACTCCCAGGAG
AGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCT
GAGCAAAGCAGACTACGAGAA
ACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGA
GCTTCAACAGGGGAGAGTGT

3.1 Signal sequences

Antibodies of the present invention maybe produced as a fusion protein with a heterologous signal sequence having a specific cleavage site at the N terminus of the mature protein. The signal sequence should be recognised and processed by the host cell. For prokaryotic host cells, the

signal sequence may be an alkaline phosphatase, penicillinase, or heat stable enterotoxin II leaders. For yeast secretion the signal sequences may be a yeast invertase leader, α factor leader or acid phosphatase leaders see e.g. WO90/13646. In mammalian cell systems, viral secretory leaders such as herpes simplex gD signal and a native immunoglobulin signal sequence are available. Typically the signal sequence is ligated in reading frame to DNA encoding the antibody of the invention.

3.2 Origin of replication

Origin of replications are well known in the art with pBR322 suitable for most gram-negative bacteria, 2μ plasmid for most yeast and various viral origins such as SV40, polyoma, adenovirus, VSV or BPV for most mammalian cells. Generally the origin of replication component is not needed for mammalian expression vectors but the SV40 may be used since it contains the early promoter.

3.3 Selection marker

Typical selection genes encode proteins that (a) confer resistance to antibiotics or other toxins e.g. ampicillin, neomycin, methotrexate or tetracycline or (b) complement auxiotrophic deficiencies or supply nutrients not available in the complex media. The selection scheme may involve arresting growth of the host cell. Cells, which have been successfully transformed with the genes encoding the therapeutic antibody of the present invention, survive due to e.g. drug resistance conferred by the selection marker. Another example is the so-called DHFR selection marker wherein transformants are cultured in the

presence of methotrexate. CHO cells are a particularly useful cell line for the DHFR selection. A further example is the glutamate synthetase expression system (Lonza Biologics). A suitable selection gene for use in yeast is the trp1 gene, see Stinchcomb *et al* Nature 282, 38, 1979.

3.4 Promoters

Suitable promoters for expressing antibodies of the invention are operably linked to DNA/polynucleotide encoding the antibody. Promoters for prokaryotic hosts include phoA promoter, Beta-lactamase and lactose promoter systems, alkaline phosphatase, tryptophan and hybrid promoters such as Tac. Promoters suitable for expression in yeast cells include 3-phosphoglycerate kinase or other glycolytic enzymes e.g. enolase, glyceralderhyde 3 phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose 6 phosphate isomerase, 3-phosphoglycerate mutase and glucokinase. Inducible yeast promoters include alcohol dehydrogenase 2, isocytochrome C, acid phosphatase, metallothionein and enzymes responsible for nitrogen metabolism or maltose/galactose utilization.

Promoters for expression in mammalian cell systems include viral promoters such as polyoma, fowlpox and adenoviruses (e.g. adenovirus 2), bovine papilloma virus, avian sarcoma virus, cytomegalovirus (in particular the immediate early gene promoter), retrovirus, hepatitis B virus, actin, rous sarcoma virus (RSV) promoter and the early or late Simian virus 40. Of course the choice of promoter is based upon suitable compatibility with the host cell used for expression. In one embodiment therefore there is provided a first plasmid comprising a RSV and/or SV40 and/or CMV promoter, DNA encoding light chain V region (VL) of the invention, κC region together with neomycin and ampicillin resistance selection markers and a second plasmid comprising a RSV or SV40

promoter, DNA encoding the heavy chain V region (VH) of the invention, DNA encoding the γ1 constant region, DHFR and ampicillin resistance markers

3.5 Enhancer element

Where appropriate, e.g. for expression in higher eukaroytics, an enhancer element operably linked to the promoter element in a vector may be used. Suitable mammalian enhancer sequences include enhancer elements from globin, elastase, albumin, fetoprotein and insulin. Alternatively, one may use an enhancer element from a eukaroytic cell virus such as SV40 enhancer (at bp100-270), cytomegalovirus early promoter enhancer, polyma enhancer, baculoviral enhancer or murine IgG2a locus (see WO04/009823). The enhancer is preferably located on the vector at a site upstream to the promoter.

3.6 Host cells

Suitable host cells for cloning or expressing vectors encoding antibodies of the invention are prokaroytic, yeast or higher eukaryotic cells. Suitable prokaryotic cells include eubacteria e.g. enterobacteriaceae such as *Escherichia* e.g. *E.Coli* (for example ATCC 31,446; 31,537; 27,325), *Enterobacter, Erwinia, Klebsiella Proteus, Salmonella* e.g. *Salmonella typhimurium*, Serratia e.g. *Serratia marcescans* and *Shigella* as well as Bacilli such as *B.subtilis* and *B.licheniformis* (see DD 266 710), Pseudomonas such as *P.aeruginosa* and *Streptomyces.* Of the yeast host cells, *Saccharomyces cerevisiae, schizosaccharomyces pombe*, Kluyveromyces (e.g. ATCC 16,045; 12,424; 24178; 56,500), yarrowia (EP402, 226), *Pichia Pastoris* (EP183, 070, see also Peng *et al*

J.Biotechnol. 108 (2004) 185-192), *Candida, Trichoderma reesia* (EP244, 234*), Penicillin, Tolypocladium* and *Aspergillus* hosts such as *A.nidulans* and *A.niger* are also contemplated.

Although Prokaryotic and yeast host cells are specifically contemplated by the invention, preferably however, host cells of the present invention are vertebrate cells. Suitable vertebrate host cells include mammalian cells such as COS-1 (ATCC No.CRL 1650) COS-7 (ATCC CRL 1651), human embryonic kidney line 293, baby hamster kidney cells (BHK) (ATCC CRL.1632), BHK570 (ATCC NO: CRL 10314), 293 (ATCC NO.CRL 1573), Chinese hamster ovary cells CHO (e.g. CHO-K1, ATCC NO: CCL 61, DHFR-CHO cell line such as DG44 (see Urlaub *et al*, (1986) Somatic Cell Mol.Genet.12, 555-556)), particularly those CHO cell lines adapted for suspension culture, mouse sertoli cells, monkey kidney cells, African green monkey kidney cells (ATCC CRL-1587), HELA cells, canine kidney cells (ATCC CCL 34), human lung cells (ATCC CCL 75), Hep G2 and myeloma or lymphoma cells e.g. NS0 (see US 5,807,715), Sp2/0, Y0.

Thus in one embodiment of the invention there is provided a stably transformed host cell comprising a vector encoding a heavy chain and/or light chain of the therapeutic antibody or antigen binding fragment thereof as hereinbefore described. Preferably such host cells comprise a first vector encoding the light chain and a second vector encoding said heavy chain.

Bacterial fermentation

Bacterial systems are particularly suited for the expression of antibody fragments. Such fragments are localised intracellularly or within the periplasma. Insoluble periplasmic proteins can be extracted and refolded to form active proteins according to methods known to those skilled in the

art, see Sanchez *et al* (1999) J.Biotechnol. 72, 13-20 and Cupit PM *et al* (1999) Lett Appl Microbiol, 29, 273-277.

3.7 Cell Culturing Methods.

Host cells transformed with vectors encoding the therapeutic antibodies of the invention or antigen binding fragments thereof may be cultured by any method known to those skilled in the art. Host cells may be cultured in spinner flasks, roller bottles or hollow fibre systems but it is preferred for large scale production that stirred tank reactors are used particularly for suspension cultures. Preferably the stirred tankers are adapted for aeration using e.g. spargers, baffles or low shear impellers. For bubble columns and airlift reactors direct aeration with air or oxygen bubbles maybe used. Where the host cells are cultured in a serum free culture media it is preferred that the media is supplemented with a cell protective agent such as pluronic F-68 to help prevent cell damage as a result of the aeration process. Depending on the host cell characteristics, either microcarriers maybe used as growth substrates for anchorage dependent cell lines or the cells maybe adapted to suspension culture (which is typical). The culturing of host cells, particularly invertebrate host cells may utilise a variety of operational modes such as fed-batch, repeated batch processing (see Drapeau *et al* (1994) cytotechnology 15: 103-109), extended batch process or perfusion culture. Although recombinantly transformed mammalian host cells may be cultured in serum-containing media such as fetal calf serum (FCS), it is preferred that such host cells are cultured in synthetic serum –free media such as disclosed in Keen *et al* (1995) Cytotechnology 17:153-163, or commercially available media such as ProCHO-CDM or UltraCHO™ (Cambrex NJ, USA), supplemented

where necessary with an energy source such as glucose and synthetic growth factors such as recombinant insulin. The serum-free culturing of host cells may require that those cells are adapted to grow in serum free conditions. One adaptation approach is to culture such host cells in serum containing media and repeatedly exchange 80% of the culture medium for the serum-free media so that the host cells learn to adapt in serum free conditions (see e.g. Scharfenberg *K et al* (1995) in *Animal Cell technology: Developments towards the 21st century* (Beuvery E.C. *et al* eds), pp619-623, Kluwer Academic publishers).

Antibodies of the invention secreted into the media may be recovered and purified using a variety of techniques to provide a degree of purification suitable for the intended use. For example the use of therapeutic antibodies of the invention for the treatment of human patients typically mandates at least 95% purity, more typically 98% or 99% purity. In the first instance, cell debris from the culture media is typically removed using centrifugation followed by a clarification step of the supernatant using e.g. microfiltration, ultrafiltration and/or depth filtration. A variety of other techniques such as dialysis and gel electrophoresis and chromatographic techniques such as hydroxyapatite (HA), affinity chromatography (optionally involving an affinity tagging system such as polyhistidine) and/or hydrophobic interaction chromatography (HIC, see US 5, 429,746) are available. In one embodiment, the antibodies of the invention, following various clarification steps, are captured using Protein A or G affinity chromatography followed by further chromatography steps such as ion exchange and/or HA chromatography, anion or cation exchange, size exclusion chromatography and ammonium sulphate precipitation. Typically, various virus removal steps are also employed (e.g. nanofiltration using e.g. a DV-20 filter). Following these various steps, a purified (preferably monoclonal) preparation comprising at least 75mg/ml

or greater e.g. 100mg/ml or greater of the antibody of the invention or antigen binding fragment thereof is provided and therefore forms an embodiment of the invention. Suitably such preparations are substantially free of aggregated forms of antibodies of the invention.

4. Pharmaceutical Compositions

Purified preparations of antibodies of the invention (particularly monoclonal preparations) as described *supra*, may be incorporated into pharmaceutical compositions for use in the treatment of human diseases and disorders such as those outlined above. Typically such compositions comprise a pharmaceutically acceptable carrier as known and called for by acceptable pharmaceutical practice, see e.g. Remingtons Pharmaceutical Sciences, 16th ed, (1980), Mack Publishing Co. Examples of such carriers include sterilised carrier such as saline, Ringers solution or dextrose solution, buffered with suitable buffers to a pH within a range of 5 to 8. Pharmaceutical compositons for injection (e.g. by intravenous, intraperitoneal, intradermal, subcutaneous, intramuscular or intraportal) or continuous infusion are suitably free of visible particulate matter and may comprise between 0.1ng to 100mg of antibody, preferably between 5mg and 25mg of antibody. Methods for the preparation of such pharmaceutical compositions are well known to those skilled in the art. In one embodiment, pharmaceutical compositions comprise between 0.1ng to 100mg of therapeutic antibodies of the invention in unit dosage form, optionally together with instructions for use. Pharmaceutical compositions of the invention may be lyophilised (freeze dried) for reconstitution prior to administration according to methods well known or apparent to those skilled in the art. Where embodiments of the invention comprise antibodies of the invention with an IgG1 isotype, a chelator of copper such as citrate (e.g. sodium citrate) or EDTA or histidine may be added to pharmaceutical

composition to reduce the degree of copper-mediated degradation of antibodies of this isotype, see EP0612251.

Effective doses and treatment regimes for admnistering the antibody of the invention are generally determined empirically and are dependent on factors such as the age, weight and health status of the patient and disease or disorder to be treated. Such factors are within the purview of the attending physican. Guidance in selecting appropriate doses may be found in e.g. Smith *et al* (1977) Antibodies in human diagnosis and therapy, Raven Press, New York. In one embodiment, the dosing regime for treating a human patient afflicted with RA is 100mg or thereabout (i.e. between 50mg to 200mg) of antibody of the invention (or antigen binding fragment thereof) administered subcutaneously per week or every two weeks. Compositions of the present invention may also be used in prophylatically.

Depending on the disease or disorder to be treated, pharmaceutical compositions comprising a therapeutically effective amount of the antibody of the invention may be used simultaneously, separately or sequentially with an effective amount of another medicament such as an anti-inflammatory agent for example a NSAID, methotrexate, bucillamine, sodium thiomalate or one or more of an anti-TNF alpha treatment such as Enbrel™ (etanercept), Remicade™ (infliximab), Humira™ (adalimumab) and/or CDP870. Antibodies of the invention maybe used in combination with an effective amount of an anti-TNF-alpha receptor antibody, see Davis MW *et al* (2000) Ann Rheum Dis 59(Suppl 1): 41-43. In other contemplated embodiments, antibodies of the invention maybe used in combination with an effective amount of an agent directed against; IL-1/IL-1R (e.g. Kineret™), CTLA4-Ig, IL-6 (see Choy *et al*, (2002) Ann.Rheum.Dis

61(suppl 1): 54), IL-8, IL-15, VEGF, IL-17, IL-18 (see Taylor *et al* (2001) Curr.Opin.Immunol.13: 611-616), anti-ICAM and/or anti-CD4 antibodies, agents directed against a member of the MMP family e.g. MMP-1, 2,3 and/or 13. Antibodies of the invention may also be used in combination with an agent that ablates cells known to be involved in the inflammatory process, e.g. CD20 positive B cells using for example Mabthera™. Other therapies in combination with antibodies of the invention include anti-angiogenic therapies such as antagonists of the integrin $\alpha_V\beta_3$, Kringles 1-5 (see Sumariwalla P *et al* (2003), Arthritis Res Ther 5:R32-R39.), soluble Flt-1 (see Miotla *et al*, (2000) Lab.Invest. 80:1195-1205) or an anti-COX-2 agent. Conveniently, a pharmaceutical composition comprising a kit of parts of the antibody of the invention or antigen binding fragment thereof together with such another medicaments optionally together with instructions for use is also contemplated by the present invention.
The invention furthermore contemplates a pharmaceutical composition comprising a therapeutically effective amount of monoclonal therapeutic antibody or antigen binding fragment thereof as hereinbefore described for use in the treatment of diseases responsive to modulation of the interaction between Site II OSM and gp130. Also contemplated is a pharmaceutical composition comprising a therapeutically effective amount of a monoclonal therapeutic antibody which antibody comprises a heavy and light chain as herein before described. Further contemplated is a pharmaceutical composition comprising a first antibody or antigen binding fragment thereof which interacts with (e.g. binds to) Site II of hOSM and a second antibody or antigen binding fragment thereof which interacts with (e.g. binds to) Site III of hOSM.

5. Clinical uses.

Antibodies of the invention may be used to treat a variety of diseases or disorders responsive to treatment that modulates the interaction between Site II of hOSM and gp130. Particular mention is made of diseases or disorders characterised by the breakdown or destruction of cartilage, particular articular cartilage. As described in detail *supra*, antibodies of the invention may be used in the treatment of inflammatory arthropathies such as RA either as a monotherapy or in combination with another treatment for such arthropathy. Antibodies of the invention may be used to treat a clinically established form of the disease in question or to prevent onset in susceptible patients or to slow or halt the progress of the disease towards clinical significance. For the treatment of RA, antibodies of the invention maybe used to prevent relapse once remission from the disease has occured. Where the patient is afflicted with an intermittent form of the disease, antibodies of the invention may be used to prolong the time interval between acute phases of the disease. Antibodies of the invention may also be used to treat the extra-articular manifestations of RA, e.g Feltys syndrome and/or to treat the formation of atherosclerotic plaques. For the treatment of RA, combinations of antibodies of the invention together with medicaments described *supra* may be used. Other arthritic diseases that may benefit from the administration of an antibody of the invention include Juvenile Onset arthritis, psoriatic arthritis and ankylosing spondylitis.

Osteoarthritis (OA) is a chronic, degenerative disease of unknown origin characterised by the gradual loss of articular cartilage and joint function. It is classified currently into two groups. Primary OA maybe localised or generalised, the latter more commonly found in post-menopausal women, with the development of Heberdens nodes. Secondary OA has an underlying cause such as trauma, obesity, Paget's disease or inflammatory arthritis. Loss of articular cartilage is often accompanied by

hypertrophic bone changes with osteophyte formation, subchrondral bone thickening and inflammation of the synovial membrane. Of particular concern is the disability afflicted to weight bearing joints such as the knee, hands and hip. OA is an extremely debilitating disease that at its severest requires joint replacement to restore mobility and to stop joint pain. Osteoarthritis of the hip has been divided into hypertrophic and atrophic forms (see Solomon L (1976) J Bone Joint Surg 58, 176) on the basis of a patients tendency to develop large osteophytes; other joints may respond similarly to the presence of the diease. Hypertrophic OA maybe associated with pyrophosphate crystal deposition and diffuse idiopathic skeletal hyperostosis. Current treatments include the use of nonopioid analgesics such as acetaminophen, and Tramadol, NSAIDS such as a Cox-2 specific inhibitor e.g. celecoxib, rofecoxib, opioid analgesics and glucosamine and chondroitin sulphate. Thus in one embodiment of the invention there is provided a method of treating osteoarthritis (e.g primary or secondary) in a human patient afflicted with such disease, which method comprises administering to said patient a therapeutically effective amount of a therapeutic antibody or fragment thereof of the invention as hereinbefore described. The invention also contemplates a combination of the therapeutic antibody of the invention together with another treatment particularly one or more of the treatments of OA described above.

Psoriasis is a chronic skin disease with significant morbidity that affects approximately 2% of the Caucasian population. While for many it may be a relatively mild disease, it can have profound effects on those affected. The disability of hospital treated patients with psoriasis has been shown to be similar to that of patients with angina and approaches that of patients with cardiac failure (Finlay *et al*, (1990); Br.J.Dermatol, 123, 751). The

commonest form of psoriasis is chronic plaque disease. This presents as well-defined red scaly plaques typically distributed over the scalp, lower back and extensor aspects of the limbs. Clinical variants include guttate psoriasis, sebopsoriasis and pustular forms of the disease. A minority of patients also develops seronegative inflammatory arthritis. Microscopically, lesional skin shows increased proliferation and abnormal differentiation of keratinocytes, infiltration by activated T-helper lymphocytes and neutrophils and activation of the cutaneous vasculature. These changes correspond to overexpression of growth factors and their receptors, proinflammatory cytokines and angiogenic peptides. However, despite intensive investigation the aetiology and pathogenesis of this disease remains obscure although a central role played by activated T cells has been demonstrated in animal model systems (see Nickoloff *et al* (1999) Arch.Dermatol.135, 546-552). Current treatments include topical treatments such as Vitamin D analogues, corticosteroids, dithranol, and retinoids such as Tazarotene gel. Phototherapy includes the use of ultraviolet B or psoralen and ultraviolet A, and excimer lasering. Systemic retinoid treatments include Etretinate and acitretin, isotretinoin, liarozole. Other treatments include methotrexate, hydroxyurea, cyclosporin and calcineurin antagonists, 6-thioguanine, azathioprine, sulfasalazine and fumaric acid esters. More recently, biological treatments such as Ontak™, Zenapax™ (Daclizumab), Basiliximab, anti-CD4 antibodies, Efalizumab, Alefacept™, Siplizumab, IDEC-114 and BMS 188667 (CTLA4Ig) have been proposed or demonstrated to be useful in the treatment of this disease. Antibodies of the present invention therefore may be used in the treatment of psoriasis (chronic plaque, guttate, sebopsoriasis, pustular, seronegative inflammatory arthritis associated psoriasis) either as a monotherapy or in combination with these treatments described *supra*. Evidence for the role of OSM in psoriatic lesions is found in Boifati *et al* (1998) Arch.Dermatol. Res 290:9,13. Antibodies of the present invention

therefore may be used in the treatment of psoriasis (chronic plaque, guttate, sebopsoriasis, pustular, seronegative inflammatory arthritis associated psoriasis), atopic dermatitis/eczema, acne, ichythosis, pemphigus, viral warts either as a monotherapy or in combination with these treatments described *supra*.

OSM has been detected in the bronchoaveolar lavage fluid of patients during acute lung injury, particularly in cases of pneumonia (Tamura S *et al* (2002) Develop Dyman 225:327-331). Neutrophils appear to be the cellular source of OSM in these patients, and OSM concentrations in the BAL fluid correlate with PMN numbers. Since neutrophils are a source of OSM, and upon activation secrete OSM, it is probable that OSM will be present in the lungs of any patient where neutrophils are a significant component of airway inflammation, including COPD and severe asthma. In addition, OSM is also expressed by (mouse) tissue eosinophils and could be a significant source of OSM during inflammation see Tamura *ibid*).

Overexpression of OSM in mouse airways using an adenoviral vector induced profound eosinophilic inflammation and matrix deposition (see Langdon C *et al* (2003) J.Immunol. 170:548-555 and also TIMP-1 expression (see Kerr C *et al* (1999) J.Interfer.Cytokine Res., 19:1195-1205. Exposure of mouse lung fibroblasts to OSM stimulated release of eotaxin, a potent eosinophil chemoattractant. Moreover, OSM stimulates the proliferation, induces collagen production and prevents apoptosis of human lung fibroblasts (see Scaffidi, A.K. *et al* (2002) Brit.J.Pharamcol 136:793-801). Although the mechanisms behind these observations are unknown, matrix deposition could, in part, be the result of a strong, specific upregulation of α_1 proteinase inhibitor synthesis (see Cichy, J. *et al* (1998) Biochem.J 329:335-339. OSM has also been found to promote

fibroblast dependent mast cell proliferation and a marked increase in histamine content (see Gyotoku E *et al* (2001) Arch.Dermatol.Res 293:508-514). Direct instillation of OSM in isolated rat lungs induced rapid and sustained IL-6 secretion (see Li, H.L. (2002) J.Drug Targ 10:55-62. Thus the present invention contemplates the use of antibodies of the invention (either as a monotherapy or in combination with another medicament) in the treatment of inflammatory lung diseases such as asthma and COPD (chronic obstructive pulmonary disorder).

OSM has been detected in the brains of MS patients, where it localises to microglia, astrocytes and infiltrating leukocytes (see Ruprecht K *et al* Journal of Neuropathology & Experimental Neurology. 60(11): 1087-98, 2001 Nov). OSM induces IL-6 and MCP-1 secretion from cerebral endothelial cells, and addition of TNFα with OSM causes a synergistic response. OSM also induces ICAM1 expression on cerebral microvascular endothelial cells, which could enhance leukocyte infiltration into brain tissue (Ruprecht K *et al ibid*). In addition to promoting inflammation in the brain, OSM may directly contribute to neuron loss. HIV patients monocyte supernatants cause profound inhibition of neuroblast growth and also neuronal cell death, and the mediator of these effects was shown to be Oncostatin M. Since many HIV patients suffer from brain atrophy caused by neuronal cell loss, OSM may be one mediator of this pathology. Clearly, OSM could also play a role in other CNS diseases where neuronal loss occurs. Interestingly in Alzheimer's disease (AD), α_1 antichymotrypsin (ACT) is one of the amyloid associated proteins and its expression is dramatically increased in disease areas, perhaps facilitating deposition of abnormal proteins in amyloid plaques and neutrofibrillary tangles. OSM, which is known to be secreted by both infiltrating activated T cells and monocytes, and microglia, is a potent inducer of ACT, and could thereby contribute to the AD pathology (see Kordula T *et al* (1998) J

Biol.Chem. 273:4112-4118 and Kordula T Journal of Neuroscience. 20(20): 7510-6, 2000).

Work by Tamura *et al* suggest that OSM may be involved in the development and maintenance of neuropathic pain (see Tamura S. *et al* (2003) Eur.J.Neurosci. 17:2287-2298). Their studies revealed a subset of nociceptive sensory neurons that express the OSMβ receptor. All the OSMβR +ve neurons also expressed VR1 and P2X3 receptors, which have been shown to be crucial for development of both neuropathic and inflammatory pain (see Jarvis M.F. *et al* (2002) PNAS 99:179-184 and Walker K.M *et al* (2003) J. Pharmacol. Exp. Ther 304, 56-62).

Thus the present invention also contemplates the use (either as a monotherapy or in combination with another medicament) of antibodies of the invention in the treatment of central nervous system diseases or disorders such as described *supra* such as multiple sclerosis (MS), Alzheimer's disease (AD) and furthermore contemplates the use in the treatment of pain, particularly neuropathic and inflammatory pain. Also OSM-/- mice have reduced noxious responses to chemical, thermal, visceral and mechanical pain, correlating with a reduction of $VR1^{+ve}$ $P2X3^{+ve}$ small neurons (see Morikawa, Y. *et al* (2004): J Neurosci 24, 1941-1947).

OSM is found in tissue macrophages in atherosclerotic lesions (see Modur V. *et al* J.Clin Invest. 100, 158-168) and as an angiogenic factor may promote the neovascularisation characteristic of atherosclerotic plaques that is thought to contributes to vessel wall fragility. As well as the angiogenic response, OSM causes induction of both IL-6 secretion in endothelial cells, where its effects are additive or synergistic with IL-1 and

TNFα respectively, and COX-2 expression (see Brown J.T *et al* (1991) J.Immunol.147: 2175-2180). Endothelial cell induction of COX2 is necessary for the angiogenic properties of OSM (see Brown J.T *et al, ibid*). However, OSM also induces expression other angiogenic factors in endothelial cells; VEGF (Vasse, M *et al* (1999) Arterioscler Thromb Vasc Biol. 19:1835-1842) and bFGF (Wijelah E.S.*et al* (1997) J.Cell Sci 110:871-879) Interestingly, human endothelial cells have about 10-20 fold greater OSM receptor density than other cells (see Modur V. *et al ibid*).

In addition to effects on endothelium, OSM also induces IL-6 and COX-2 expression in vascular smooth muscle cells (VSMC) as well as causing striking changes in cell morphology (Bernard C. *et al* (1999) Circ.Res. 85:1124-1131). Calcium deposits are usually found in advanced atherosclerotic lesions where macrophages are the predominant inflammatory cell. Macrophges are a major source of OSM and interestingly, this cytokine can induce bone-type alkaline phosphatase and calcium deposition in VSMC cultures (Shioi A. *et al* (2002) Circ.Res. 91:9-16). OSM also respectively induces and depresses tissue factor (TF) and TF pathway inhibitor (TFPI) secretion from VSMCs, resulting in a potent procoagulant activity in VSMC culture supernatants (Mirshahi F. *et al* (2002) Blood Coag.Fibrinol. 13:449-455). Furthermore, OSM affects von-Willebrand factor, tissue-type plasminogen activator and PAI-1 secretion from endothelial cells in a way that suggests that "OSM could play a key role in the development of atherosclerotic lesions" (Portau J *et al* (1998) Blood Coag.Fibrinol. 9,609-615).

Plasma levels of fibrinogen are an important vascular risk factor and OSM is a potent inducer of fibrinogen secretion in studies with a hepatoma cell line (Vasse.M *et al* (1996) Haemostasis 26, Suppl 4, 331-339). However, at high concentrations (50 ng/ml) OSM also increased human LDL

receptor expression (Liu *et al* (2003) Aterio.Thromb.Vasc.Biol.23: 90-96). Finally, OSM promotes cholesterol esterification in J774 monocyte-macrophages, and may therefore contribute to this process during Foam cell development in atherosclerotic lesions (Maziere C *et al* (1996) Biochem. Biophys Acta 1300, 30-34).

Thus the present invention contemplates the use of antibodies of the invention in the treatment of diseases or disorders of the cardiovascular system. Also contemplated is use of antibodies of the invention in the treatment of atherosclerosis and diseases of endothelial cell origin. Further contemplated is the use of antibodies of the invention in treating patients afflicted with HIV, particularly to treat conditions resulting from infection with the virus such as Karposi sarcoma.

Antibodies of the invention may also be used in diseases of cell cycle regulation e.g. cancer (such as prostate cancer), myeloma.

Although the present invention has been described principally in relation to the treatment of human diseases or disorders, the present invention may also have applications in the treatment of similar diseases or disorders in non-human mammals.

The present invention is now described by way of example only.

Exemplification

1. Generation of monoclonal antibodies

Monoclonal antibodies are produced by hybridoma cells generally in accordance with the method set forth in (E Harlow and D Lane, Antibodies a Laboratory Manual, Cold Spring Harbor Laboratory, 1988. The result of

the fusion of mouse myeloma cells with B-lymphocytes from mice immunised with the target antigen. The hybridoma cell is immortalised by the myeloma fusion partner while the capacity to produce antibodies is provided by the B lymphocyte.

Four SJL mice were immunised by intraperitoneal injection with glycosylated human OSM (hOSM) produced in CHO cells suspended in RIBI adjuvant (Sigma). The mice were boosted with hOSM only after 2 weeks then with hOSM neutralised with anti-site III monoclonal antibody (OM4/11.17; OSM: Mab 1:1.5 wt: wt) to drive the immune response towards Site II after a further 2 weeks then again with the OSM-MAb complex after another 2.5 weeks and finally with OSM only after 5 weeks. Three months after initial immunisation, spleens were removed and B lymphocytes fused with mouse myeloma cells derived from P3X cells using PEG1500 (Boehringer) to generate hybridomas. Individual hybridoma cell lines were cloned by limiting dilution (E Harlow and D Lane). Wells containing single colonies were identified microscopically and supernatants tested for activity. Cells from the most active clones were expanded for cryopreservation, antibody production etc.
Initial OSM antibody selection was on the basis of specificity and potency in neutralising human glycosylated OSM assessed in the gp130 inhibition ELISA and the KB cell assay, (see below) the latter providing a check of OSM specificity. After identification of antibodies of sufficient potency and correct specificity, further selection criteria were applied:
1/ cross-reactivity against cynomolgus monkey OSM
2/ maintenance of activity against human OSM in the presence of pooled human AB serum
3/ maintenance of activity against a human neutrophil OSM library and against RA synovial fluid cell–derived OSM

1920 hybridomas were screened in the gp130 inhibition ELISA. 43 gave more than 50% inhibition and limited dose response experiments were done on 15 from which 6 were selected for further study. These were subcloned and master clones were selected.

Antibody (clone 10D3) was selected on the basis of potency. It will be immediately apparent to those skilled in the art that an antibody that interacts with Site III of hOSM may be found by determining whether a candidate antibody inhibits or blocks the interaction between hOSM and OSMR.

2. CLONING OF VARIABLE REGIONS OF CLONE 10D3

Total RNA was extracted from clone 10D3 hybridoma cells and the cDNA of the heavy and light variable domains was produced by reverse transcription using primers specific for the murine leader sequence and the antibody constant regions according to the pre-determined isotype (IgG1/κ). The cDNA of the variable heavy and light domains was then cloned into vector pCR2.1 for sequencing.

2.1 RNA extraction

Total RNA was extracted from pellets of 10^6 cells of hybridoma clone 10D3 using the SV Total RNA Isolation System from Promega according to manufacturer's instructions.

2.2 Reverse transcription

RNA was reverse transcribed to produce cDNA of the variable heavy and light domains using primers specific for the murine leader sequences and murine IgGγ2a/κ constant regions. The mixture of primers used is set forth in Jones ST and Bendig MM Bio/technology 9:88-89 (1991)

Pools of murine V_H and V_L leader sequence forward primers were prepared at 50μM. Solutions of the murine γ2a and κ constant region reverse primers were also prepared at 50μM.

2.3 Reverse Transcription PCR (RT-PCR)

Reverse transcription of the RNA encoding the variable heavy and light regions was carried out in duplicates using the Access RT-PCR System from Promega according to manufacturer's instructions. VH and VL forward and reverse primers were as described above.

3. Cloning of PCR product of 2.3

3.1 Gel purification

The products of RT-PCR ($2xV_H$ and $2xV_L$) were loaded in gel loading solution on a preparative 1% agarose gel containing 0.01% ethidium bromide and run in TAE buffer at 100V for 1hour and the V region bands excised. A 100bp DNA ladder was also run on the gel to allow identification of the V_H and V_L bands.

The DNA fragments were extracted and purified from the gel using the QIAquick ™Gel extraction kit from Qiagen according to manufacturer's instructions.

3.2 Ligation

The purified RT-PCR fragments ($2xV_H$ and $2xV_L$) were cloned into the pCR2.1 vector using the TA cloning kit from Invitrogen according to manufacturer's instructions.

3.3 Transformation

Ligated plasmids were transformed into TOP10F' cells according to TA cloning kit instructions. 50µl and 200µl of transformed cells were spread on L-agar plates containing 100µg/ml ampicillin and coated with 8µl of 500mM IPTG solution and 16µl of 50mg/ml X-Gal solution in DMF. Plates were incubated overnight at 37°C.

3.4 Sequencing

5 white colonies were cultured overnight at 37°C in 5ml LB medium supplemented with 100µg/ml ampicillin.
pCR2.1 plasmids containing *10D3* V_H and VL domains were extracted and purified using the Qiagen QIAprep Spin Miniprep kit according to manufacturer's instructions.
The V_H and VL domains were sequenced using primers T7, M13 for and M13 rev.
10D3 $_{VH}$ domain amino acid sequence (consensus of 10 clones from 2 RT-PCR reactions):

EVQLQQSGPELVKPGASVKISCKASGYIFTDYNMDWVKQSHGKKLEWIGDINPNN
GGTIDNQKFKDKATLTVDKSSSTAYMELRSLTSEDTAVYYCARGIYYYGSHYFDY
WGQGTTLTVSS

10D3 VL domain amino acid sequence (consensus of 10 clones from 2 RT-PCR reactions):

QIVLTQSPAIMSASPGEKVTMTCSATSSVSVMHWFQKKSGTSPKRWIYDTSKLAS
GVPTRFSGSGSGTSYSLTISSMEAEDTATYYCQQWSSNPLTFGSGTKLELK

4. Chimaeric antibody

A chimaeric antibody consisting of parent murine V regions of 3.4 grafted onto human IgG1/k wild type C regions was designed to confirm the cloning of the correct murine V regions and also to be used as a reference when testing humanised constructs. The chimaeric antibody was expressed in CHO cells, purified and tested for affinity to OSM site II in the gp130 inhibition ELISA and KB cell assay.

The cloned murine V regions were amplified by PCR to introduce restriction sites required for cloning into mammalian expression vectors Rld and Rln. Hind III and Spe I sites were designed to frame the V_H domain and allow cloning into a modified Rld vector containing the human γ1 wild type C region. Hind III and BsiW I sites were designed to frame the V_L domain and allow cloning into a modified Rln vector containing the human κ C region.

4.1 PCR amplification

V_H forward primer:

The Hind III restriction site is underlined and Kozak sequence in bold.

VH forward: 5'-GAT GAA GCT T**GC CAC** CAT GGG ATG GAG CTG GGT CTT T-3'

VH reverse: 5'-GAT GGA CTA GTG TGC CTT GGC CCC AAT A-3'

The Spe I restriction site is underlined.

V_L forward primer:

VL forward: 5'-GAT GAA GCT T**GC CAC** CAT GGA TTT ACA GGT GCA GAT T-3'

The Hind III restriction site is underlined and Kozak sequence in bold.

VL reverse: 5'-GAT GCG TAC GTT TCA GCT CCA GCT TGG TCC C-3'

The BsiW I restriction site is underlined

PCR reaction:	water	66µl
	10x PCR buffer	10µl
	dNTP (2mM)	10µl
	primer 1 (5µM)	4µl
	primer 2 (5µM)	4µl
	AmpliTaq polymerase	2µl
	purified plasmid	4µl
	total vol	100µl

Primer 1: V_H or V_L forward primer

Primer 2: V_H or V_L reverse primer

Purified plasmid: pCR2.1 V_H or V_L plasmid purified by Qiagen Minipreps (diluted 200x)

PCR cycle:
1- 95°C for 4min
2- 95°C for 1min
3- 55°C for 1min
4- 72°C for 1min
5- 72°C for 7min

steps 2 to 4: were repeated 30 times

4.2 Cloning into mammalian expression vectors

The PCR products were purified using the MinElute PCR Purification kit from Qiagen according to manufacturer's instructions.

4.2.1 Restriction digests

The V_H PCR product and RId hCγ1wt mammalian expression vector were digested Hind III-Spe I:

10x buffer (NEBuffer2)	5µl
BSA 100x (NEB)	0.5µl
DNA	5µl
Hind III (Promega)	2µl
Spe I (NEB)	2µl
water	35.5µl
total vol	50µl

DNA: purified V_H PCR product or RId hCγ1wt vector (at 0.25mg/ml)
Incubated at 2h at 37°C.

The V_L PCR product and RIn hCκ mammalian expression vector were digested Hind III-BsiW I:

10x buffer (NEBuffer2)	5µl
DNA	5µl
Hind III (Promega)	2µl
water	38µl
total vol	50µl

DNA: purified V_L PCR product or RIn hCκ vector (at 0.25mg/ml)
Incubated at 2h at 37°C.
2µl of BsiW I (NEB) was added and incubated 2h at 55°C.

4.2.2 Gel purification

The products of restriction digests were loaded in gel loading solution on a preparative 1% agarose gel containing 0.01% ethidium bromide and run in TAE buffer at 100V for 1hour and the RId and RIn vector as well as V_H and

V_L PCR fragment bands were excised. A 100bp DNA ladder was also run on the gel to allow identification of the V_H, V_L and vector bands.
The DNA was extracted and purified from the gel using the QIAquick Gel extraction kit from Qiagen according to manufacturer's instructions.

4.2.3 Ligation

The V_H PCR fragment Hind III-Spe I digested was ligated into the Rld hCγ1wt vector Hind III-Spe I digested.
The V_L PCR fragment Hind III-BsiW I digested was ligated into the Rln hCκ vector Hind III-BsiW I digested.
The ligation was carried out using the LigaFast Rapid DNA Ligation System from Promega according to manufacturer's instructions providing:

V_H: vector: Rld hCγ1wt Hind III-Spe I digested
insert: V_H PCR fragment Hind III-Spe I digested
V_L: vector: Rln hCκ Hind III-BsiW I digested
insert: V_L PCR fragment Hind III-BsiW I digested

4.2.4 Transformation

Ligated products were transformed into DH5α competent cells:
200µl DH5α vials were thawed on ice.
50µl aliquots were prepared in transformation tubes.
2µl of ligation mixture was added and mixed gently with a pipette tip followed by incubation for 30min on ice.
The mixture was incubated for 45sec at 42°C without shaking.
This was then transferred to ice for 2min.
450µl SOC medium was added and the tubes incubated for 1h at 37°C on shaker incubator.

100µl of culture was spread on L-agar plates supplemented with 100µg/ml ampicillin and incubated overnight at 37°C.

4.2.5 Sequencing

VH and VL clones were cultured overnight at 37°C in 5ml LB medium supplemented with 100µg/ml ampicillin.
Rld and Rln plasmids containing V_H and VL domains respectively were extracted and purified using the QIAprep Spin Miniprep kit from Qiagen according to manufacturer's instructions.
The VH region was sequenced using forward primers in the Rld vector and signal sequence and reverse primer in the human Cγ1 region.

The VL region was sequenced using forward primers in the Rln vector and signal sequence and reverse primer in the human Cκ region.

Clones with the correct V_H and V_L sequences were identified and plasmids prepared for expression in CHO cells.

4.3 Chimaeric antibody expression in CHO cells

Rld and Rln plasmids containing 10D3 V_H and V_L domains respectively were transiently co-transfected into CHO cells and expressed. The chimaeric antibody produced was purified from cell culture supernatant by affinity chromatography on rProtein A Sepharose and its affinity for OSM was evaluated in the gp130 inhibition ELISA and KB cell assay (see below).

4.3.1 Plasmid purification

DH5α cells containing Rld-10D3V_H and Rln-10D3V_L plasmids were cultured in 5ml of LB media supplemented with 100μg/ml ampicillin for 8h at 37°C in a shaker incubator.

200ml of LB media supplemented with 100μg/ml ampicillin was inoculated with 1ml of day culture and incubated overnight at 37°C in a shaker incubator.

The plasmids were extracted and purified using the QIAfilter Plasmid Maxi kit from Qiagen according to manufacturer's instructions. The ethanol pellet was resuspended in 200μl TE buffer and plasmid concentration was measured by absorbance at 260nm after 100-fold dilution of stock solution.

4.3.2 Transfection

CHO cells were cultured to confluence in Dulbecco's MEM with Glutamax-1 (DMEM) media supplemented with Ultra Low Fetal Bovine Serum and 1% Penicillin-Streptomycin in 4x175cm^2 BD Falcon tissue culture flasks at 37°C.

For each flask, in a 50ml Falcon tube,the following were added and mixed:

8ml Optimem 1 with Glutamax-1

20μg Rld-10D3V_H purified plasmid

20μg Rln-10D3V_L purified plasmid

240μl TransFast Transfection Reagent under vortex

The mixture was incubated for 10-15min at RT.

DMEM media was removed from flask then the mixture was vortexed and added to flask.

The mixture was incubated at 37°C for 1h.

32ml Optimem was added to the flask and incubated at 37°C for 48-72h.

4.3.3 Purification of chimaeric antibody

Media from all 175cm^2 flasks were pooled and centrifuged at 1500rpm for 3min on an MSE Mistral 2000 and supernatant passed through a 500mL Filter System 0.22μm CA.

The antibody was purified from clarified supernatant on an Amersham Biosciences Akta Explorer using Unicorn software.

The column used was a 1ml HiTrap rProtein A Sepharose FF.

The flow rate was 1ml/min.

The column was equilibrated with 10CV of Dulbecco's PBS then loaded with clarified supernatant through pump A.

The column was washed with 20CV of Dulbecco's PBS, pump A was washed to waste and a further 10CV of Dulbecco's PBS was passed through the column to ensure complete clearance of supernatant.

The antibody was eluted with 10CV of ImmunoPure IgG Elution Buffer (Pierce) and collected in 1ml fractions containing 100μl of 1M Trizma-HCl pH8.0 neutralisation buffer.

The column was re-equilibrated with 5CV of Dulbecco's PBS.

Antibody in eluate fractions was quantified by reading the absorbance at 280nm against a blank solution containing 10 volumes of ImmunoPure IgG Elution Buffer + 1 volume of 1M Trizma-HCl pH8.0 and fractions with sufficient amounts of pure antibody were pooled and stored in 100μl aliquots at –20°C.

4.4 Analysis of chimaeric antibody

The 10D3 chimaeric antibody was analysed in the gp130 inhibition ELISA and KB cell assay for their potency in neutralising both human and cynomolgus OSM.

Protocols for the gp130 inhibition ELISA and KB cell assay are set forth below.

10D3 chimaeric antibodies neutralise OSM in the gp130 inhibition ELISA and KB cell assay

These results confirm that the correct variable regions have been cloned successfully to produce an antigen binding chimaeric antibody capable of binding both human and cynomologus OSM site II.

The 10D3 variable heavy and light domains can now be humanised.

The murine variable regions were cloned and sequenced then grafted onto human γ1/k constant regions to produce a chimeric antibody. The chimeric 10D3 antibody showed potency against human and cynomolgus OSM equivalent to that of the parent murine antibody in the gp130 ELISA and KB cell assays (see below).

The murine antibody was humanised using the "best fit" strategy.

For the variable heavy domain, a sequence with 65% identity was selected and the murine CDRs grafted onto the human frameworks. A number of constructs were designed with various backmutations in the frameworks to recover affinity. These constructs are:

Construct	Backmutations
A	T28I
B	T28I, R71V, T73K
C	T28I, V67A, M69L, R71V, T73K
D	T28I, M48I, G44K, V67A, M69L, R71V, T73K

For the variable light domain, a sequence with 60.0% identity was selected and the murine CDRs grafted onto the human frameworks. A

number of constructs were designed with various backmutations in the frameworks to recover affinity. These constructs are:

Construct	Backmutations
LA	none (straight graft)
LB	L46R, L47W
LC	Y36F, Q38K
LD	Y36F, Q38K, L46R, L47W
LE	Y36F, Q38K, L46R, L47W, F71Y

Only the least and most backmutated constructs (A, D, LA, LE) were synthesised by build up of overlapping oligos. Four humanised antibody combinations (ALA, ALE, DLA, DLE) were expressed at small scale in CHO cells and the supernatant analysed for antibody affinity in the gp130 ELISA.

Only humanised antibodies ALE and DLE showed inhibition in the gp130 ELISA but the inhibition by ALE was not sufficient because of the low concentration of antibody in the supernatant so DLE was selected. Production of humanised antibody DLE was scaled up in CHO cells and the antibody purified and analysed in the gp130 ELISA and KB cell assay using 10D3 chimeric antibody as control.

IC50 values (gp130 ELISA) (μg/ml):

	hOSM	cOSM
chimera	0.032	0.246
DLE	0.021	0.059

Humanised antibody 10D3 DLE is at least as potent if not more potent than the chimeric antibody against human OSM and cynomolgus OSM in the gp130 ELISA.

Humanised 10D3 DLE and 10D3 chimeric antibodies were analysed in the KB cell assay.10D3 DLE gave IC50 values of 0.205μg/ml against human OSM and 0.07μg/ml against cynomolgus OSM.

In conclusion, anti-human OSM site II antibody 10D3 has been successfully humanised and shows potency equivalent to that of the parent murine antibody.

Materials

SV Total RNA Isolation System: Promega Z3100
Access RT-PCR System: Promega A1250
QIAquick Gel Extraction kit: Qiagen 28704
Gel loading solution: Sigma G7654
Agarose: Invitrogen 15510-019
Ethidium bromide: Sigma E1510
TAE buffer: in-house
100bp DNA ladder: New England BioLabs N3231S
TA cloning kit: Invitrogen 45-0046
TOP10F' cells: Invitrogen 44-0300
L-agar + 100μg/ml ampicillin: in-house
X-Gal, 50mg/ml in DMF: Promega V394A
AmpliTaq DNA Polymerase: Applied Biosystems
10x PCR buffer: Applied Biosystems
E-Gel 1.2% agarose: Invitrogen G501801

LB medium + 100μg/ml ampicillin: in-house
QIAprep Spin Miniprep kit: Qiagen 27106
MinElute PCR Purification kit: Qiagen 28004
NEBuffer2 10x conc: New England Biolabs B7002S
Purified BSA 100x conc: New England Biolabs B9001S
BsiW I: New England Biolabs R0553L
Hind III: Promega R604A
Spe I: New England Biolabs R0133S
LigaFast Rapid DNA Ligation System: Promega M8225
MAX Efficiency DH5α Chemically Competent cells: Invitrogen 18258-012
SOC media: in-house
QIAfilter Plasmid Maxi kit: Qiagen 12263
Dulbecco's MEM with Glutamax-1: Invitrogen 31966-021
Optimem 1 with Glutamax-1: Invitrogen 51985-026
TransFast Transfection Reagent: Promega E2431
1ml HiTrap rProtein A Sepharose FF: Amersham Biosciences 17-5079-01
Dulbecco's PBS: Sigma D8537
ImmunoPure IgG Elution Buffer: Pierce 21009
1M Trizma-HCl pH8.0: Sigma T2694
ProofStart DNA Polymerase: Qiagen 1016816
ProofStart PCR buffer: Qiagen 1016961

7. gp130 inhibition ELISA

OSM binds sequentially to gp130 and either the OSM receptor or LIF receptor. The assay described here allows measurement of OSM (for example hOSM) bound to gp130 on an ELISA plate. In addition, the assay allows the measurement of inhibition of OSM binding to the gp130 receptor by antibodies raised against OSM site II.

7.1 Materials

1. Nunc Immunoplate 1 F96 Maxisorp (Life Technologies, 4-39454A)
2. Human gp130-Fc 100µg/ml (R&D Systems, 671-GP-100)
3. PBS
4. BSA (Sigma A7030)
5. Human recombinant OSM 10µg/ml (R&D Systems)
6. Biotinylated anti human OSM 50µg/ml (R&D Systems, BAF295)
7. Streptavidin HRP (Amersham RPN4401)
8. TMB (Sigma)
9. Sulphuric acid
10. Tween 20 (Sigma P7949)

7.2 Preparation of reagents

1. **Preparation of plates:** Dilute the human gp130-Fc to 1µg/ml in PBS. Add 50µl/well, cover and incubate overnight at 4°C.
2. Wash buffer: to 1L PBS add 500µl Tween 20 (0.05%)
3. Blocking buffer: to 500ml PBS add 5g BSA (1%)

7.3 Method

1. Wash plate using standard plate washer protocol and tap dry.
2. Add 200µl/well **blocking buffer** and incubate for 1 hour at RT.
3. Wash as in step 1.
4. Add 50µl/well OSM standard or sample. Cover and agitate for 2 hours at RT.
   (OSM is diluted to 100, 50, 25, 12.5, 6.25, 3.125, 1.563 and 0 ng/ml in blocking buffer or tissue culture medium depending on the sample)
5. Wash as in step 1.
6. Add 50µl/well biotinylated anti human OSM diluted to 30ng/ml in blocking buffer. Cover and agitate for 1 hour at RT.
7. Wash as in step 1.

8. Add 50µl/well streptavidin HRPdiluted 1/4000 in blocking buffer. Cover and agitate for 30 min. at RT.
9. Wash as in step 1.
10. Add 100µl/well TMB substrate. Cover and agitate for 30 minutes at room temperature.
11. Add 50µl/well 1M H_2SO_4.
12. Read OD 450_{nm}.

7.4 Use of assay for analysis of antibody mediated inhibition of gp130-OSM binding.

1) Mix 25 ng/ml OSM with various concentrations of anti-OSM antibody, or various dilutions of antisera containing OSM antibodies. Incubate for 1h at RT.
2) Add 50µl/ well of the antibody-OSM mixture to a 96 well plate containing bound gp130, prepared as above.
3) Proceed with assay as described above.

8. KB assay

Introduction

KB cells (a human epithelial cell line) express mRNA for gp130 together with LIF and OSM receptors (Mosley, J. Biol Chem., 271 (50) 32635-32643). Both OSM and LIF induce IL-6 release from KB cells. This cell line has been used to identify monoclonal antibodies modulating the interaction between OSM and gp130.

8.1 Method

KB cells were obtained from ECACC (Accession no 94050408) and maintained in DMEM + 10% heat inactivated FCS, supplemented with glutamine ("KB medium"). Cells grow as a monolayer and were split twice weekly. Sigma non-enzymatic cell dissociation medium or Versene was used to detach the cells.

1. Add $2x10^4$ cells/ 100μl/ well/ 96 well plate and incubate overnight (37°C, 5% CO_2).
2. Make up OSM standards in culture media
3. Make up 1ng/ml OSM + antibody / sera dilutions. Incubate for 1h at RT.
4. Carefully remove media from KB cell plate and add OSM standards and OSM-antibody mixtures.
5. Incubate for ~16-18h at 37°C.
6. Remove culture medium and assay for IL-6.

Note:

- Culture medium can be kept frozen until ready for analysis.
- Culture medium should be diluted ~20 fold for assay.
- In screening hybridomas, the ratio of cloning medium to KB medium should be constant, and the OSM standards should be made up in this mixture.
- Stimulation of KB cells with ~100 ng/ml OSM gives maximal IL-6 output, but 1 ng/ml is sufficient to look for antibody neutralising activity.

9. Competition Assay.

This assay allows the measurement of inhibition of binding of the humanised antibody having a heavy chain of SEQ.I.D.NO: 11 and a light

chain having a light chain of SEQ.I.D.NO: 12 (for the purpose of this example denoted as 10D3-DLE) to soluble glycosylated hOSM by a candidate non-human antibody that specifically binds to Site II of hOSM.

The plate is coated with anti-site III monoclonal antibody (referred to herein as OM4-11.31).
For the standard curve: 10D3-DLE purified standard serially diluted from 1μg/ml is incubated with soluble glycosylated human OSM at 50ng/ml. The antibody binds to OSM through site II and the complex is then captured on the plate by the primary antibody against site III.
For the competition assay: the candidate antibody serially diluted from 1μg/ml is incubated with soluble glycosylated human OSM at 50ng/ml and 10D3-DLE at 150ng/ml.
The presence of complexed 10D3-B3L2 is detected by an anti-human gamma chain secondary antibody.

Method:

1/ Coating

A Nunc Maxisorp Immunoplate was coated with 50μl per well of anti-human OSM site III antibody (OM4-11.31, in-house) at 4μg/ml in PBS. The plate was incubated overnight at 4°C.

2/ Blocking

The plate was washed 3 times with PBS + 0.05% Tween (PBST).
100μl of 1% BSA (Sigma A7030) in PBS was added to each well.
The plate was incubated at room temperature for 2h with shaking.

3/ Pre-incubation

10D3DLE standard:

A solution of 10D3-DLE antibody at 1μg/ml in 50ng/ml human OSM in block buffer was prepared and 67μl added to 2 wells in row A of a non-adsorbent 96-well plate. The antibody was serially diluted 1:3 in 50μl of 50ng/ml human OSM in block buffer from row B to G.

Competing antibody:

A solution of competing antibody at 1μg/ml in 150ng/ml 10D3-DLE + 50ng/ml hOSM in block buffer was prepared and 100μl added to 2 wells in row A of a non-adsorbent 96-well plate. The antibody was serially diluted 1:1 in 50μl of 150ng/ml 10D3-DLE + 50ng/ml human OSM in block buffer from row B to G. Two wells were incubated with diluent without competing antibody.

The pre-incubation plate was incubated at room temperature for 1h under static conditions.

4/ Incubation

The coated plate was washed 3 times with PBST.

45μl of each standard and sample was transferred from the pre-incubation plate to equivalent wells on the coated plate. PBS was added to blank wells.

The plate was incubated at room temperature for 2h under shaking.

5/ Secondary antibody

The plate was washed 3 times with PBST.

50μl of goat anti-human γ chain-peroxidase (Sigma A6029) diluted 2000 fold in block buffer was added to each well.

The plate was incubated at room temperature for 1h under shaking.

6/ Substrate

The plate was washed 3 times with PBST.

The OPD substrate (Sigma P9187) was prepared in water according to manufacturer's instructions.
50µl was added to each well.
The plate was incubated at room temperature.

7/ Stop
Once the coloration had sufficiently developed, the chromogenic reaction was stopped by addition of 10µl of 3M H_2SO_4 per well.
The plate was read at 490nm in a plate reader using blank wells as 0 absorbance.

The standard curve of absorbance at 490nm against 10D3 concentration was plotted.
The complexed 10D3 concentration in the samples containing competing antibody was read off the standard curve. % inhibition was calculated as:

100- [(10D3 conc in sample in ng/ml ÷ 150ng/ml) x 100]

The curve of % inhibition against competing antibody concentration was plotted and the % inhibition of 10D3 at equimolarity of competing antibody (150ng/ml of competing antibody) was read off the curve.

Claims

1. A therapeutic antibody that specifically binds to OSM, particularly hOSM, and modulates the interaction between OSM and gp130.

2. The antibody according to claim 1 comprising a CDRH3 of SEQ.I.D.NO: 3.

3. The antibody of claim 2 further comprising;

CDRH1 of SEQ.I.D.NO: 1
CDRH2 of SEQ.I.D.NO: 2
CDRL1 of SEQ.I.D.NO: 4
CDRL2 of SEQ.I.D.NO: 5
CDRL3 of SEQ.I.D.NO: 6.

4. The antibody according to any one of claims 1 to 3 wherein the antibody is selected from the group consisting of; intact, chimaeric, humanised, bispecific, heteroconjugate.

5. The antibody according to any one of claims 1 to 4 wherein the antibody is an intact antibody.

6. The antibody of claim 5 wherein the intact antibody is murine, rat, rabbit, primate or human.

7. The antibody of claim 6 wherein the intact antibody is human.

8. The antibody of claim 4 wherein the antibody is chimaeric or humanised.

9. The antibody of claim 8 wherein the antibody is humanised.

10. The antibody of claim 9 wherein residues 28,44,48,67,69,71,73 of the human acceptor heavy chain framework region and positions 36,38,46,47,71 of the human acceptor light chain framework are substituted by the corresponding residues in the donor antibody framework from which CDRH3 is derived.

11. A humanised therapeutic antibody or antigen binding fragment thereof which specifically binds to hOSM wherein said antibody or fragment thereof comprises CDRs of SEQ.I.D.NO: 1,2,3,4,5 and 6 wherein the human heavy chain framework comprises one or more (e.g. all) of the following residues (or a conservative substitute thereof):

Position	**Residue**
28	I
48	I
44	K
67	A
69	L
71	V
73	K

and the human light chain comprises one or more (e.g.all) of the following residues (or conservative substitute thereof);

Position	**Residue**
36	F
38	K

46	R
47	W
71	Y

12. A humanised therapeutic antibody comprising a VH domain of SEQ.I.D.NO: 9 and a VL domain of SEQ.I.D.NO: 10.

13. A humanised therapeutic antibody comprising a heavy chain of SEQ.I.D.NO: 11 and a light chain of SEQ.I.D.NO: 12.

14. A therapeutic antibody according to any one of claims 1 to 12 further comprising a human constant region.

15. A therapeutic antibody according to claim 14 wherein the constant region is of an IgG isotype e.g. IgG1 or IgG4.

16. The therapeutic antibody of any preceding claim wherein said antibody modulates the interaction between Site II of hOSM and gp130.

17. A therapeutic antibody of claim 16 wherein said antibody inhibits said interaction.

18. The antibody of claim 17 wherein said antibody blocks said interaction.

19. An antigen binding fragment of the therapeutic antibody of any preceding claim.

20. A fragment according to claim 19 wherein said fragment is selected from the group consisting of; Fab, Fab', $F(ab)_2$, ScFv.

21. A pharmaceutical composition comprising a therapeutic antibody or antigen binding fragment thereof according to any preceding claim.

22. A method of treating a human patient afflicted with a disease or disorder responsive to modulation of the interaction between hOSM and gp130, said method comprising the step of administering to said patient a therapeutically effective amount of the composition of claim 21.

23. A method of treating a human patient afflicted with a chronic inflammatory disease or disorder said method comprising the step of administering to said patient a therapeutically effective amount of the composition of claim 21.

24. A method of treating a human patient afflicted with an arthritic disease or disorder said method comprising the step of administering to said patient a therapeutically effective amount of the composition of claim 21.

25. A method according to claim 24 wherein said patient is afflicted with rheumatoid arthritis and/or osteoarthritis.

26. A method of treating a human patient afflicted with an inflammatory lung disease such as asthma or COPD, said method comprising the step of administering to said patient a therapeutically effective amount of the composition of claim 21.

27. A method of treating a human patient afflicted with psoriasis, said method comprising the step of administering to said patient a therapeutically effective amount of the composition of claim 21.

28. A method of treating a human patient afflicted with a cardiovascular disease or disorder such as atherosclerosis which method comprising the step of administering to said patient a therapeutically effective amount of the composition of claim 21.

29. A method of treating a human patient afflicted with Karposi sarcoma which method comprising the step of administering to said patient a therapeutically effective amount of the composition of claim 21.

30. Use of a therapeutic antibody or antigen binding fragment of any one of claims 1 to 20 in the manufacture of a medicament for the treatment of a disease responsive to modulation of the interaction between hOSM and gp130 such as rheumatoid arthritis, osteoarthritis, asthma, COPD.

31. A medicament comprising the therapeutic antibody or antigen binding fragment of any one of claims 1 to 20.

32. A vector (e.g. plasmid) encoding the heavy chain and/or light chain of the therapeutic antibody or antigen binding fragment of any one of claims 1 to 20, for example said vector comprises a nucleotide of any one of claims 33 to 36.

33. A polynucleotide encoding the VH domain of SEQ.I.D.NO: 9 said polynucleotide consisting essentially of) SEQ.I.D.NO:17.

34. A polynucleotide encoding the VL domain of SEQ.I.D.NO: 10, said polynucleotide consisting essentially of) SEQ.I.D.NO: 18.

35. A polynucleotide encoding the heavy chain of SEQ.I.D.NO: 11, said polynucleotide consisting essentially of) SEQ.I.D.NO: 19.

36. A polynucleotide encoding the light chain of SEQ.I.D.NO: 12, said polynucleotide consisting essentially of) SEQ.I.D.NO: 20.

37. A stably transformed host cell comprising the vector of claim 32.

38. The host cell of claim 37 wherein said host cell is vertebrate cell.

39. The host cell of claim 38 wherein said cell is mammalian.

40. The host cell of claim 39 wherein said cell is adapted for serum-free culturing.

41. The host cell of claim 39 or 40 wherein said cell is adapted for suspension culturing.

42. The host cell of any one of claims 39 to 41 wherein said cell is CHO or NS0.

43. A process for the manufacture of a therapeutic antibody or antibody fragment of any of claims 1 to 20 comprising the step of culturing a host cell of claims 37 to 42.

44. The process of claim 43 wherein said host cell is suspension cultured in serum free media.

45. An antibody or antigen binding fragment which competitively inhibits the binding of the therapeutic antibody of any one of claims 2, 3, 12 or 13 to OSM, particularly hOSM, more particularly Site II of hOSM.

46. A pharmaceutical composition comprising the competing antibody of claim 45.

47. A method of treating a human patient afflicted with a disease or disorder responsive to modulation of the interaction between hOSM and gp130 (such as an arthritic disease e.g. rheumatoid arthritis and/or osteoarthritis) which method comprises the step of administering to said patient a therapeutically effective amount of the composition of claim 46.

48. A method of provoking a mammal (e.g.non-human such as mouse/rat/rabbit) to mount an immune response (e.g. B cell upregulation) biased towards a first epitope of an administered polypeptide which method comprises administering to said mammal an effective amount of said polypeptide coupled to an antigen binding moiety (e.g. antibody) specific for a second epitope.

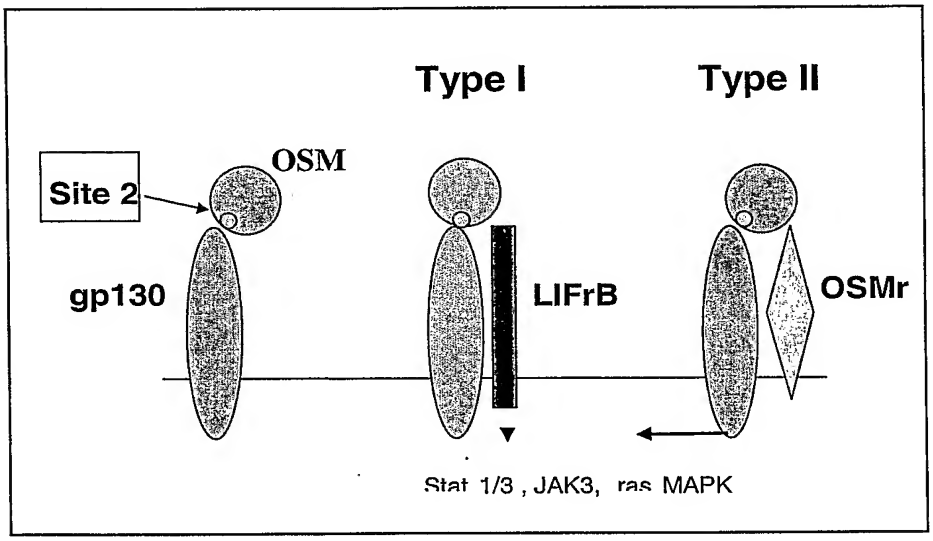
49. The method of claim 48 wherein said polypeptide is a cytokine (e.g. soluble cytokine such as OSM).

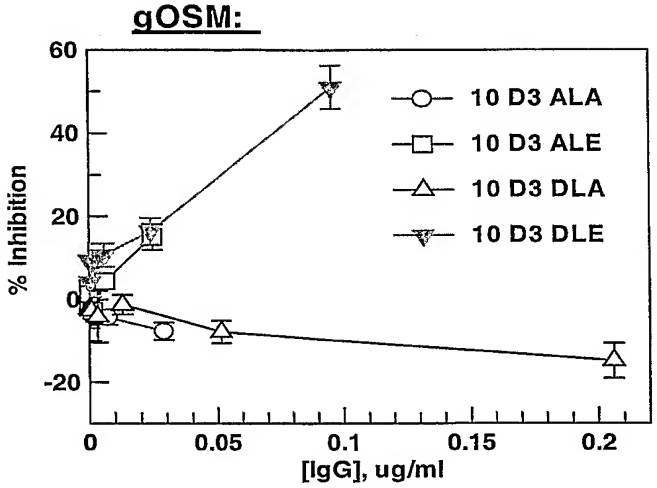
50. The method of claim 48 or 49 wherein said mammal is immunised and/or boosted (i.e. second or subsequent administered) with said polypeptide coupled to an antigen binding moiety.

51. The method of any one of claims 48 to 50 wherein said first and second epitopes interact with a signalling interacting partner.

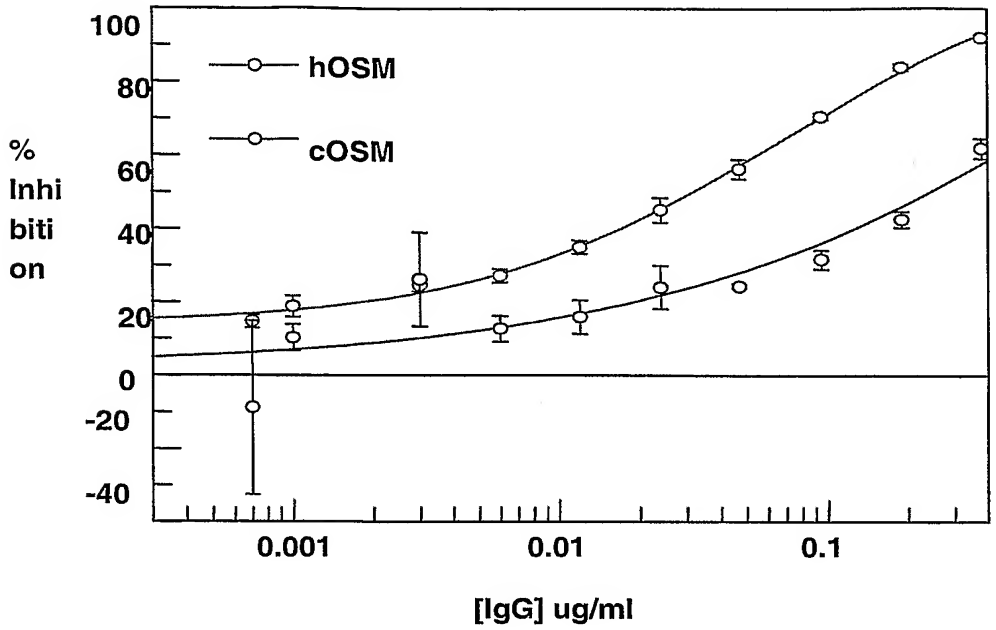
52. The method of claim 51 wherein said first and second epitopes interact with different signalling interacting partners.

Fig.1


Type I
Type II
Site 2
OSM
gp130
LIFrB
OSMr
Stat 1/3 , JAK3, ras MAPK


gOSM:
60
40
20
0
-20
% Inhibition
0
0.05
0.1
0.15
0.2
[IgG], ug/ml
10 D3 ALA
10 D3 ALE
10 D3 DLA
10 D3 DLE

10D3 Chimera



10D3 DLE